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ON THE COVER

A photograph of a Caspian horse, an Iranian breed of a small horse of Oriental type. In this issue, seasonal changes in serum progesterone levels have been studied in Caspian mares; see page 21.

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TABLE OF CONTENTS

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Magnetized water as an alternative strategy to improve the poultry production system

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ABSTRACT

There is a demand for new alternative strategies to improve the health and production of poultry. One of these approaches is the magnetization of the drinking water. Therefore, this review discussed the different effects of magnetized water on poultry production traits, carcass traits, immune response, antimicrobial activity, blood parameters, and oxidative stress. Exposure of water to diverse magnetic fields positively rearranges the chemical structure and consequently improves its quality. Broilers that received magnetized water showed an improvement in the body weight gain and feed conversion ratio. Layers revealed an increase in egg quantity and quality, and breeders exhibited a rise in the fertility and hatchability parameters. Improvement of the dressing and carcass traits has been shown after providing magnetized water. Moreover, magnetized water may enhance humoral immunity, decrease the pathogenic microbial load, and increase the beneficial bacterial populations. Amelioration in the liver and kidney enzymes and other blood parameters as well as relieving of oxidative stress were also detected in birds supplied with magnetized water. In conclusion, further research in this area and also more encouragement of poultry farmers to treat birds with magnetized water are recommended.

Keywords

antimicrobial, immunity, magnetized water, oxidative stress, production

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Abbreviations

E. coli: Escherichia coli
Ig: Immunoglobulin
ND: Newcastle disease
HDL: High-density lipoprotein
LDL: Low-density lipoprotein
ALT: alanine aminotransferase
AST: aspartate aminotransferase

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Introduction

The fast-growing poultry industry prohibited the use of antimicrobial growth promoters during the production period to avoid the development of antibiotic-resistant pathogens that affect poultry flock health and performance. These changes necessitate the search for alternatives in the poultry production system [1]. Poultry flock performance is affected by the quality of water provided to birds. Improper cleaning and disinfection of water lines and pipes in poultry farms result in the increased risk of water contamination which adversely affects the health and production of birds [2]. Several pathogenic bacteria, such as *Salmonella*, *E. coli*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Shigella*, *Vibrio*, *Staphylococci*, and *Streptococci* species, as well as fungi, including *Aspergillus*, *Penicillium*, and *Mucor* species, have been isolated from the drinking water in poultry farms [3-6].

In addition, using underground water in the poultry farms of some developing countries represents a major challenge. The suitability of water for poultry consumption is affected by total dissolved salts, salinity, nitrates [7], and excessive concentrations of inorganic ions, such as Ca^{+2}, Mg^{+2}, Na^{+}, Cl^{-}, SO_4^{-}, and HCO_3^{-} in water [8]. Different techniques have been applied to improve water quality, one of which involves using magnetic forces to magnetize water. Magnetized technology depends mainly on moving electric charge in the ionized form and the magnetic field [9]. For several decades, magnetized water has been used in several sectors such as agriculture, industry, medicine, environment, and veterinary practice.

Various biological characteristics could be detected after magnetizing the natural water. Magnetization of water restores natural energy [10] and increases electrical conductivity and dielectric constant which consequently improves the water quality [11, 12]. Furthermore, a magnetic field enhances the formation of a beneficial structure, raises fluidity and hydroxyl alkaline ions [13], reduces acidity, and forms alkaline molecules [14]. Magnetization costs less than other chemical and physical water treatments. In addition, it could augment the dissolving capacity for vitamins and minerals [15], leading to facilitated nutrient transfer across cell membranes, uptake, and utilization [16, 17].

The different effects of using magnetized water on the poultry production system are summarized in Figure 1. Many investigations with controversial results have been performed in different countries to evaluate the impact of magnetized drinking water on poultry production. Therefore, this review aimed to discuss the different effects of magnetized water on poultry production traits, carcass traits, immune response, antimicrobial activity, blood parameters, and oxidative stress.

Mechanism of water magnetization in poultry farms

Water is a paramagnetic element in which some or most of its single atoms, ions, or molecules have a constant magnetic dipole moment. Water magnetization can be achieved by passing water through magnetic tubes or putting a constant neodymium disc magnetic device (modifier) in water. The magnet may be neodymium, iron, or boron, and is coated with an epoxy-nickel-copper-nickel for the effective protection of the magnet against corrosion and acidic conditions. The number of magnets (funnel), the shape of magnets (liner or circle), and the diameter and thickness of magnets that create the magnetic field vary according to the manufacturer's instructions. The unit of measuring magnetic fields is teslas or gauss as one tesla is equal to 10,000 gauss. The magnetic field strength is also variable. Water is usually passed through a magnetic funnel at a relatively low speed to prevent water overflow and then, it is collected into graduated cylinders for distribution. Next, the purified water is circulated in a closed loop from a closed tank through a tubing system linked to a pump. Therefore, a solution can pass through the field many times in a closed cycle. The incubation period is usually several hours and the memory of magnetic treatment extends beyond 200 h. However, water usually retains the magnetic characteristics for 6-12 h following water passage through the magnetic field of the funnel. Because of decreasing the magnetization of water over time, the magnetization process may be repeated. Magnetized water, particularly with high gauss (more than 1000 gauss/month), can improve health, immunity, growth of broilers, and some egg production traits of hens.

The molecules of regular tap water are not separated from each other and form clusters due to the presence of hydrogen bonds. When tap water passes through a stable magnetic field, the size of clusters and the number of grouped molecules decline. Consequently, the activity of water molecules rises with better bioavailability and absorption into the cell's membranes. A magnetic field can enhance water purification and affect both the physical and chemical processes of water dissolution and crystallization. Accordingly, some positive changes in water characteristics, such as increasing the oxygen ratio, water viscosity, water salinity, and velocity of dissolved salts and amino acids may be detected. Augmenting the ratio of dissolved oxygen is attributed to decreasing the organic matter content in magnetized water. However, water salinity rises due to increased soluble salts. Other physical properties of water, such as increasing the pH, conductivity, evaporating temperature, minerals,

and organic matter, as well as reducing total bacterial count can be detected after magnetization of water. As a result of the diminished tension of magnetized water surface by 10%-12% with increasing velocity, the water penetration and diffusion into the cell wall could be facilitated and accelerated. Moreover, it has been indicated that more hydroxyl (OH-) ions are created, forming alkaline molecules and decreasing water acidity. Therefore, both the electric conductivity and dielectric constant of water will increase. Normal water has a pH of about 7, while magnetized water may show a pH of 9.2 after exposure to a 7000 gauss strength magnet for a long time. All of these effects depended on the power of the magnetized field and the duration of exposure to this field.

Effects of magnetized water on the poultry production system

The different effects of magnetized water on the production system are summarized in Table 1.

Production traits

Broilers

Water magnetization induces significant improvement in water quality which consequently reflects the production performance parameters of broilers [18, 19]. Broilers that received magnetized water showed a shorter growing period, enhanced growth rate, improved meat quality [20], and lower mortality [21]. A significant increase in the broiler body weight was observed in day-old broiler chicks treated with magnetized water for 5 weeks compared to control non-treated chickens [22-24]. In addition, magnetized water significantly enhanced body weight, weight gain, and feed efficiency during 1-35 days of age [25].

The type of feed ingredient, processing, solution, water magnetization, and ambient temperature are key factors for the acid-binding capacity of feed stuff and pH, which cause a well-known effect on the absorption of some nutrients in the intestine [26]. El-Hanoun et al. [27] demonstrated that magnetized tap water could improve the body weight and feed conversion ratio of broiler geese compared to the well water provided group. In addition, improved performance was detected in gosling's progeny which indicated a long-term carryover effect of magnetized water and magnetization on progeny performance [27]. Enhanced final body weight (7.3%), daily weight gain (7.4%), feed conversion ratio (11.7%), protein efficiency ratio (23.3%), and production index (20.6%) were reported in broiler chicks that received magnetized water [28].

Magnetization of water induces many positive changes in water quality via rearranging cations/anions in a new format in the media [19]. Increasing the concentration of oxygen and the solubility of minerals hasten the transfer of water and nutrients in the body's compartments and enhances the growth of cells. As a result, the permeability of the cell wall will be improved and the surface tension and electric conductivity will decrease [29-31]. Furthermore, magnetic treatment of water could enhance the health status of animals by reducing lime deposition and microbial load in water pipes [32].

On the other hand, some studies showed no effect of magnetized water supplementation on broilers' performance. Al-Mufarrej et al. [18] found no significant effect of magnetized water on the feed intake, feed conversion ratio, and body weight gain of broiler chickens. Cai et al. [33] demonstrated that broiler chickens fed on magnetized water showed diminished feed consumption and increased feed conversion ratio. Moreover, the performance and health of guinea fowl were not affected by magnetized drinking water [34]. Water magnetization at 500 gausses with 5, 10, and 15 min exposure time induced no significant differences in the mortality rate, feed intake, body weight, weight gain, feed conversion ratio, performance index, and viability of broiler chicks [35, 36].

The inconsistent results regarding the effect of magnetized water on broilers' production performance might be related to various factors, including the differences in the magnetic field strengths, duration of water passing through the magnetized device, mineral content, and cleanness of water before passing through the magnetic device [36].

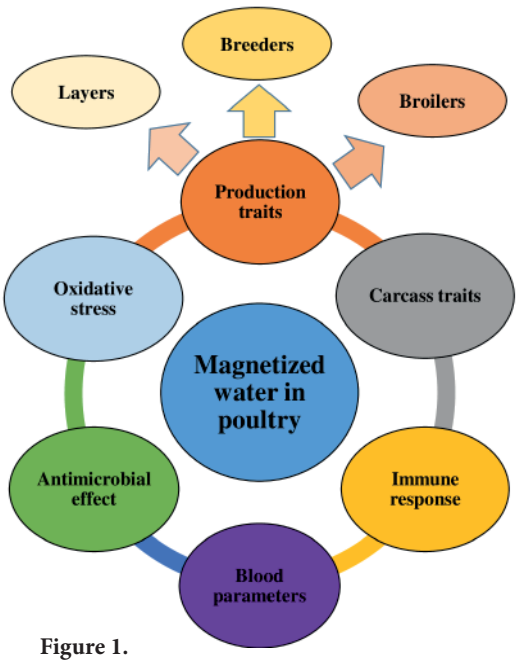

Figure 1.
The different effects of using magnetized water on the poultry production system

Table 1.
The different effects of magnetized water on production system of poultry.

Water treatment	Findings	References
Magnetized water (500 gauss) for 1-32-days-old broilers	- Decreased water consumption without influence on performance, carcass composition, and immune response. - Non-significant reduction in performance between sexes.	18
Magnetized water (6000 gauss) for broiler chickens	- Reduced mortality. - Increased feed conversion coefficient, performance efficiency factor, European production efficiency factor, and livability.	*24*
Magnetized water (85-102 tesla in a rate of 10 hrs/day) for 1-42-days-old broilers	- Increased growth performance, immune response, and lactic acid producing bacteria. - Reduced total bacterial and coliform counts.	*28*
Magnetized water (500 gauss for 5, 10, and 15 min) for 42-days-old broilers	- Improvement of productive traits (body weight, weight gain, feed intake, feed conversion ratio, mortality, viability, and production index)	35
Magnetized water (1850 gauss) for 42-days-old broilers	- No influence on feed conversion ratio, body weight gain, feed intake, and livability.	36
Magnetized water (500 gauss with different speed flow) for 1-42-days-old broilers	- Improved final body weight, daily gain, feed conversion ratio, protein efficiency ratio, and performance index, but decreased total feed intake. - Improved total protein, globulin serum concentration, antibody titer production, immune organs relative weights, improved liver functions, and dressing percent. - Reduced total intestinal bacterial and coliforms counts, but increased lactic acid count.	44
Magnetized water (more than 1000 gauss/month) for boilers and layers	- Improved immunity and growth (approximately +4 % in weight) for broilers. - Enhanced eggshell thickness (approximately +9 %) for hens.	*45*
Magnetized water (6 hrs/ day for 9 weeks) for day-old Pekin ducklings	- Improved feed conversion ratio, protein efficiency ratio, production index, and phagocytic activity. - Increased viscosity of the ileal content and intestinal villi length.	*48*
Magnetized water (13.200 gauss/6 hrs/ day) from 5-35 days of age	- Maintaining viability of the ND virus vaccine titer for 4 hrs, but minimizing E. coli and S. Typhimurium survival. - Increased performance traits, carcass and immune organ weights, total Lactobacillus count, and sera Ig concentrations, - Reduced stress markers and total bacterial and enterobacteriaceae counts.	*49*
Magnetized water every 6 hrs with acidifier 4m/liter for 1-35-days-old broilers	- Enhanced growth performance, immune response, intestinal health, and absorptive capacity.	*50*
Magnetized water (5 µ Tesla rms/ 30 min/ day) for 1-35 days-old broilers	- Protection from E. *acervulina*, E. *maxima*, and E. *tenella* infestation	*53*
Magnetized water (1000 gauss discharge 1000 liters/hr) for 36-days-old broilers	- Low blood sugar, cholesterol, and triglyceride	*54*
Magnetized water (500 or 1000 gauss for 60 days) for Japanese quail	- Increasing red blood cells, white blood cells, haemoglobin, packed cell volume percentage, total protein levels, alkaline phosphatase activity, and mitotic index. - Reduced total cholesterol, LDL, triglycerides, glutathione, and glucose levels.	*56*
Magnetized water every 12 hrs for 56-days-old broilers	- Improved final body weight, daily body weight gain, total body weight gain values, and feed conversion ratio.	*61*
Magnetized water (4000 gauss for 28 weeks) for one-year-old Egyptian geese	- Improved body weight, feed conversion ratio, and renal and hepatic functions. - Increased production, quality and hatchability of eggs, levels of reproductive hormones, and antioxidant in blood.	*27*
Magnetized water (3000 gauss) for 48-weeks-old laying hens for 2 months	- Improved internal egg quality characteristics, shell mass, and egg: feed efficiency. - No influence on egg numbers and egg mass.	*37*
Magnetized water (2000, 3000, and 4000 gauss) for 28-week-old laying hens	- Improved feed consumption, feed conversion ratio, eggs weight and mass, shell thickness, and albumen, albumen dry matter, and yolk percentages. - Increased red blood cell count, haemoglobin, serum phosphorus level, blood pH, and triiodothyronine. - Decreasing albumin/ globulin ratio.	*38*
Magnetized water (0.65 tesla) for 32-weeks-old laying hens	- The limestone crystals tended to be larger and more uniform in size with increased egg strength.	*41*
Magnetized water (4000 or 6000 gauss) for one-year-old geese	- Improved semen quality, reproductive traits, functions of kidney and liver, antioxidant status, and immune response.	*46*

Table 1 cont.

Magnetized water (4000 gauss) for rabbit bucks	-Increasing body weight, feed intake, reaction time, fertility, sperm concentration, mass motility, total live sperm, testosterone hormone, IgA, and antioxidant enzymes. - Decreasing lipid peroxidation biomarker malondialdehyde and thiobarbituric acid-reactive substances.	*17*
Magnetized water (4000 gauss) for weaning rabbits	- Improved body weight, body weight gain, feed conversion ratio, and feed intake. - Increasing red blood cells counts, hemoglobin concentration, packed cells volume, and white blood cells count. - High lymphocyte and monocyte percentages, IgG, IgM, and IgA, plasma total protein, and globulin concentrations - Low neutrophil, eosinophils, and basophils percentages, plasma total lipid, cholesterol creatinine, aspartate amino transferase, and alanine amino transferase concentrations.	*47*
Magnetized water (1200 and 3600 gauss for 30 days) for 6-weeks-old rabbits	- Improved productive performance and functions of liver and kidneys	*52*
Magnetized water (450 to 500 gauss) for Zealand albino rabbits	- Improved oxygenated hemoglobin derivative-oxyhemoglobin. - Decreased oxidized form of hemoglobin (methemoglobin).	*59*

Layers

Magnetic water treatment is now widely used in poultry farms to enhance the production performance of layer chickens [14, 37]. Gimmizah layer chickens that received 2000, 3000, and 4000 gausses of magnetized water showed higher feed intake, better feed conversion ratio, and an increase in egg weight, shell thickness, yolk, albumin percentage, and albumin dry matter compared to the non-treated control birds [38]. The authors attributed these positive effects on the performance and egg quality to the higher levels of triiodothyronine induced by magnetic water treatment. Studies by Roland and Harms [39] and Verma [40] revealed that magnetized water may help in increasing calcium solubility and its precipitation in bones when egg shell calcification occurs. Moreover, egg shell quality may enhance due to the potential influence of water magnetization on the availability of calcium carbonate and consequently the strength of the egg shell [41].

Breeders

Mustafa and Hassani [42] observed improved production traits, egg quality, and survival rate in breeder chickens after magnetizing water in summer. Furthermore, fertility, egg quantity, quality, hatchability, and levels of reproductive hormones, such as progesterone and estrogen increased in geese treated with magnetized water in comparison with birds supplied with well water [27]. Roosters that drank magnetic water revealed more improvement in semen quantity and quality than those that received tape water [43].

Carcass traits

El-Hanoun et al. [27] indicated that treating well water with a magnetic field induced great positive effects by increasing the percentage of dressed carcass and skin but decreased the abdominal fat of geese that received this water. Moreover, Soltan et al. [44] showed that magnetized water treatment significantly raised the dressing percentage of broilers, but had no significant effect on the other carcass traits. However, no significant influence of magnetized water on the broilers carcass weight, thigh, drumstick, breast, back, neck, and abdominal fat has been reported [18]. The weight and length of the intestinal tract increased in broiler chickens treated with magnetized water for 42 days as a result of augmented villus height and muscle thickness of the jejunum [36].

Immune response

Water subjected to a magnetic field of over 1000 gauss could improve the birds' health and immunity [45]. Magnetization of water with a 6000 gauss magnetic field significantly increased the levels of serum IgG, IgM, and IgA in Egyptian male geese [46]. Similarly, treatment of water with 4000 gauss significantly raised the concentrations of IgG, IgM, and IgA in rabbits [47]. El-Katcha et al. [48] concluded that magnetic water and some additives significantly improved the phagocytosis of Pekin ducklings compared to controls. Recently, Soliman et al. [49] reported a significant rise in the total IgG and IgM against live ND virus vaccination after the treatment of broiler chickens with magnetized water. In addition, magnetized water significantly enhanced antibody titers against live ND virus vaccine in broiler chickens infected with Salmonella enteritidis [50]. Contradictory results showed that the exposure of water to a magnetic field did not influence antibody responses to sheep red blood cell antigens in broiler chickens [18]. Moreover, an in vi-

tro study showed a significant reduction in ND virus vaccine titer administered in water, saline, and magnetic water at levels of 94.13%, 84.53%, and 10.31%, respectively [49].

Antimicrobial effect

Water direct exposure to a magnetic field could inhibit the growth of *E. coli* [51]. Magnetized water *in vitro* was capable of decreasing *E. coli* O157:H7 and Salmonella typhimurium survival by 54.91% and 39.89%, respectively [49]. *In vivo* investigations revealed the inhibitory effect of magnetized water against some important pathogenic microorganisms. Broiler chickens that drank magnetized water and were challenged with *Salmonella enteritidis* showed enhanced performance, improved health and immunity, and reduced challenge organism count [50]. In addition, Soltan et al. [44] found a more significant decrease in the total intestinal bacterial count (39.3%) and coliforms count (40%), but a more remarkable increase in the lactic acid bacterial count (44.4%) and *Lactobacillus* bacteria (14.6%) in broilers treated with magnetized water compared to those of the control group. Broilers provided with magnetized water (13.200 gausses) for 6 h daily from the 5th day till the 35th day of age showed a significant reduction in the total bacterial count and total *E. coli* count in the intestinal and breast muscles which indicated the low resistance and neutralization of pathogenic microorganisms [49]. In the study of Mahmoud et al. [52], the results indicated the strong antimicrobial activity of magnetized water against gram-negative bacteria, such as *E. coli*, and beneficial effects on gram-positive ones, such as *Lactobacillus* in the gut of rabbits. Broiler chicks drank magnetized tap water, acidified water, or a combined treatment that could overcome *Salmonella enteritidis* infection hazard [50]. Moreover, the daily treatment of day-old broiler chicks with electromagnetic field-exposed water for 30 min during 35 days protected broiler chickens from *Eimeria* (*E. acervulina*), *E. maxima*, and *E. tenella* infestation, and this type of water could be considered as a possible alternative to anticoccidial medications [53].

Blood parameters

Blood parameters of poultry could be positively affected by drinking magnetized water [36]. Significant reduction in the blood levels of glucose, cholesterol, and triglycerides [54] and the elevation of protein metabolism [55] have been demonstrated in broiler chickens treated with magnetized water compared to the control birds. Similar results were obtained by Soliman et al. [49] who found that chickens supplied with magnetized water had a significant decline in glucose, creatinine, total cholesterol, triglycerides, and ALT serum concentrations. An improvement in renal function indicated by the lower levels of urea and creatinine and an enhancement of liver function shown by the lower activity of AST and ALT enzymes have been observed in the geese supplemented with magnetized tap and well water [27].

El-Katcha et al. [48] proved that magnetized water significantly raised HDL, but reduced LDL concentrations in growing Pekin ducklings compared to those of the control group. Consequently, the reduction in serum total cholesterol, triglyceride, and LDL-cholesterol level and the increase in the HDL-cholesterol level may lead to hypotriglyceridemia, beneficial raise of "good" cholesterol, and healthier birds [56]. In rabbits, a significant reduction of creatinine and liver enzymes [57] and glutathione concentration [58] were observed after receiving magnetized water compared to the control animals. Treatment with magnetized water improved hemoglobin derivatives (methemoglobin, carboxyhemoglobin, and sulfohemoglobin), resulting in the increased non-functional hemoglobin form and enhanced overall hemoglobin level [59]. Additionally, significant increases were demonstrated in the hemoglobin level, red blood cell, white blood cell count, and packed cell volume in Japanese quails that drank magnetized water [56].

Contrary results were detected by Alhammer et al. [60] who found that the mice treated with magnetized water exhibited no effect on some liver enzymes, such as oxaloacetic transaminase, serum glutamic pyruvic transaminase, and alkaline phosphatase compared to the control non-treated group. Similarly, Mahmoud et al. [61] did not report any modification in the ALT and AST enzymes in broiler chickens after receiving magnetized water. In another study on guinea fowl, magnetized water did not affect blood potassium and chloride levels [34].

Oxidative stress

The activity of superoxide dismutase increases in the magnetic field [62]. Magnetized water could significantly influence the antioxidant capacity and reduce oxidative stress indicated by a decrease in malondialdehyde and nitric oxide levels and an increase in superoxide dismutase activity in the heart, liver, and kidney in broiler and mice trials [49, 63]. El-Katcha et al. [50] reported that broiler chicks that were infected with *Salmonella enteritidis* and received magnetized water exhibited a significant rise in the total antioxidant activity and enhanced superoxide dismutase, while at the same time, hydrogen peroxide concentration was reduced.

Furthermore, a significant reduction in lipid peroxidation markers, such as malondialdehyde and thiobarbituric acid reactive substances in male rab-

bits treated with 4000 gausses magnetized water was reported [17]. In rats, magnetized water improved the antioxidant levels in type 2 diabetic animals [64, 65].

Conclusion

Magnetized water is one of the water treatment approaches that has attracted researchers and poultry producers owing to its low cost compared to other chemical and physical treatments of water. The use of magnetized water in the poultry production system could be regarded as an alternative strategy to improve the production traits of broilers, layers, and breeders, and enhance the carcass traits. In addition, water magnetization could improve immune response, antimicrobial activity, blood parameters, and oxidative stress. Magnetized water could be used to alleviate the harmful effects of medicines, toxins, and environmental pollutants on humans and animals. Therefore, further research in this area and encouragement of poultry farmers to treat birds with magnetized water are recommended.

Authors' Contributions

WAA collected the data and wrote and revised the manuscript.

Competing Interests

The author has no conflicts of interest to declare that are relevant to the content of this article.

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Molecular detection and phylogenetic analysis of *Bovicola caprae* in the west and northwest of Iran based on cytochrome oxidase 1 marker

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ABSTRACT

Lice are permanent, obligate ectoparasites for birds and mammals. *Bovicola caprae* causes hypersensitivity, irritation, dermatitis, anemia, lower weight gain, and lower productivity in goats. This study was conducted to investigate *B. caprae* by molecular methods based on the mitochondrial genome in the West and Northwest of Iran. A total of 1017 samples of chewing lice collected from ten cities in five provinces were identified using diagnostic keys. After DNA extraction and PCR, samples were sent for sequencing. Morphological results were consistent with molecular examinations. Nucleotide sequencing of samples isolated from different cities based on mitochondrial genome showed 100% intraspecific similarity. The sequences of *B. caprae* isolated in this study appeared in a branch next to the Canadian and Chinese samples in the phylogenetic tree with more than 90% similarity. The results of mitochondrial gene analysis in the present research showed that this fragment is useful for showing intraspecific similarity and species and genus differentiation of *B. caprae*.

Keywords

COX1, Iran, Lice, Molecular study

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Number of Tables: 1
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Number of Pages: 10

Abbreviations

PCR: Polymerase chain reaction
COX1: Cytochrome oxidase 1
mtDNA: Mitochondrial DNA
MBST: Molecular biological system transfer
µl: Microliter
µm: Micromolar
mM: Millimolar

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Introduction

Lice are obligate ectoparasites of birds and mammals and are classified in the order Phthiraptera. Four phthirapteran suborders are recognized: the chewing louse suborders Amblycera, Ischnocera, and Rhynchophthirina, and the sucking louse suborder Anoplura [1-3]. They are hemi-metabolic insects and spend all stages of their lives on a host [2, 4, 5]. In these four suborders, there are 24 families, 303 genera, and about 5000 species of lice, of which about 4000 species infest birds and about 1000 species infest mammals [6].

B. caprae, the goat-biting louse, has a brownish-red head and thorax, with a yellow abdomen and brown crossbands, and a truncated anterior margin of the head. Their life cycle lasts about 3-4 weeks and they spend all their lives on one host [4, 7], leading to a decrease in the quality and production of wool in goats [8]. Lice infestation is a major problem for small ruminants and causes serious damage to livestock through mortality and reduced productivity, reproduction, fertility, and skin value in the tanning industry [9]. Symptoms of lice infestation in goats include hypersensitivity to the protein in the lice saliva, which results in irritation and alopecia as the main clinical signs. Secondary infections may occur due to excessive scratching [4]. Lice can also cause hypoglycemia, hypoproteinemia, and hypoalbuminemia following the chronic loss of nutrients [10]. Moreover, lice infestation reduces productivity and reproduction in goats because of anemia and miscarriage. Reduced productivity in goats due to decreased weight gain and reproductive disorders is of considerable economic significance [4].

Lice species are conventionally identified based on morphological characteristics. However, accurate identification of species and subspecies of lice based on morphological characteristics is difficult because lice have a large variety and similar morphology. To solve this problem, researchers have used molecular markers, including mitochondrial genes and nuclear ribosomal genes. An appropriate genetic marker is a basic prerequisite for success in many evolutionary studies [11]. mtDNA is a valuable evolutionary tool for diverse structural and evolutionary aspects. These features include easy isolation, high copy number, no recombination, protected sequence and structure in metazoans, and a wide range of mutations in different molecular regions. Certain characteristics of COX1 make it a unique and suitable marker for evolutionary studies. Its size and structure have been preserved in the studied aerobic organisms, and mutation studies have mapped its reaction centers, facilitating the interpretation of sequence differences in gene function [11].

As generally agreed, lice are categorized in four suborders. In contrast, there is no agreement on the phylogenetic relationships of these groups and their classification [12]. Although phylogeny at the suborder level was suggested by Lyal in 1985, it was flawed due to the questionable monophyly of Ischnocera as a suborder [6]. Since then, many studies have addressed phylogeny at the subordinate level in lice. Johnson et al. (2002) analyzed the sequence of three genes (EF1-18S-cox1) of 21 species from four suborders and showed that Ischnocera is monophyletic [13]. In another study, Yoshizawa et al. (2003) analyzed the rrnL, rrnS sequence of 18 species and showed that Ischnocera was paraphyletic, grouping the species of Trichodectidae and Anoplura together [14]. Genetic analysis of insect species provides useful information on the taxonomic relationships, epidemiology, disease transmission, and control. Despite the importance of the economic damage of lice infestation, little genetic evaluation has been performed in this field. Therefore, to fill this gap, the *B. caprae* DNA sequence was analyzed using a cox1 mitochondrial marker for the first time in Iran.

Result

All collected specimens were of the *B. caprae* species. Table 1 shows the total number of samples collected per city. Using primers (HCO2198, LCO1490), cox1 was amplified in ten samples of a 669 bp fragment, and the PCR product was observed on 1.5% agarose gel. After sequencing the purified PCR samples of cox1, the sequences were validated in the BLAST system of NCBI and compared with the reference sequences in the GenBank. Next, they were registered in the GenBank with assigned access numbers (ID: OK135715-OK135724). The sequences were aligned using MEGA software and the ClustalW method. Phylogenetic relationships were investigated by drawing a phylogenetic tree based on the maximum composite likelihood method with a bootstrap test (1000 replications).

The phylogenetic tree showed a high similarity in the sequences of the isolates in this study. All *B. caprae* samples isolated from the studied cities were located in one sub-branch. Samples of *B. caprae* cox1 isolated from Iranian goats in this study were located next to the Chinese *B. caprae* specimen (MF927687.1). The alignment results of all isolates in this study showed that these sequences are completely similar (Figure 1). During the analyses, one of the sequences related to the city of Bahar isolate (OK135715) was compared with other similar sequences in GenBank and was examined bioinformatically (Figure 2). The phyloge-

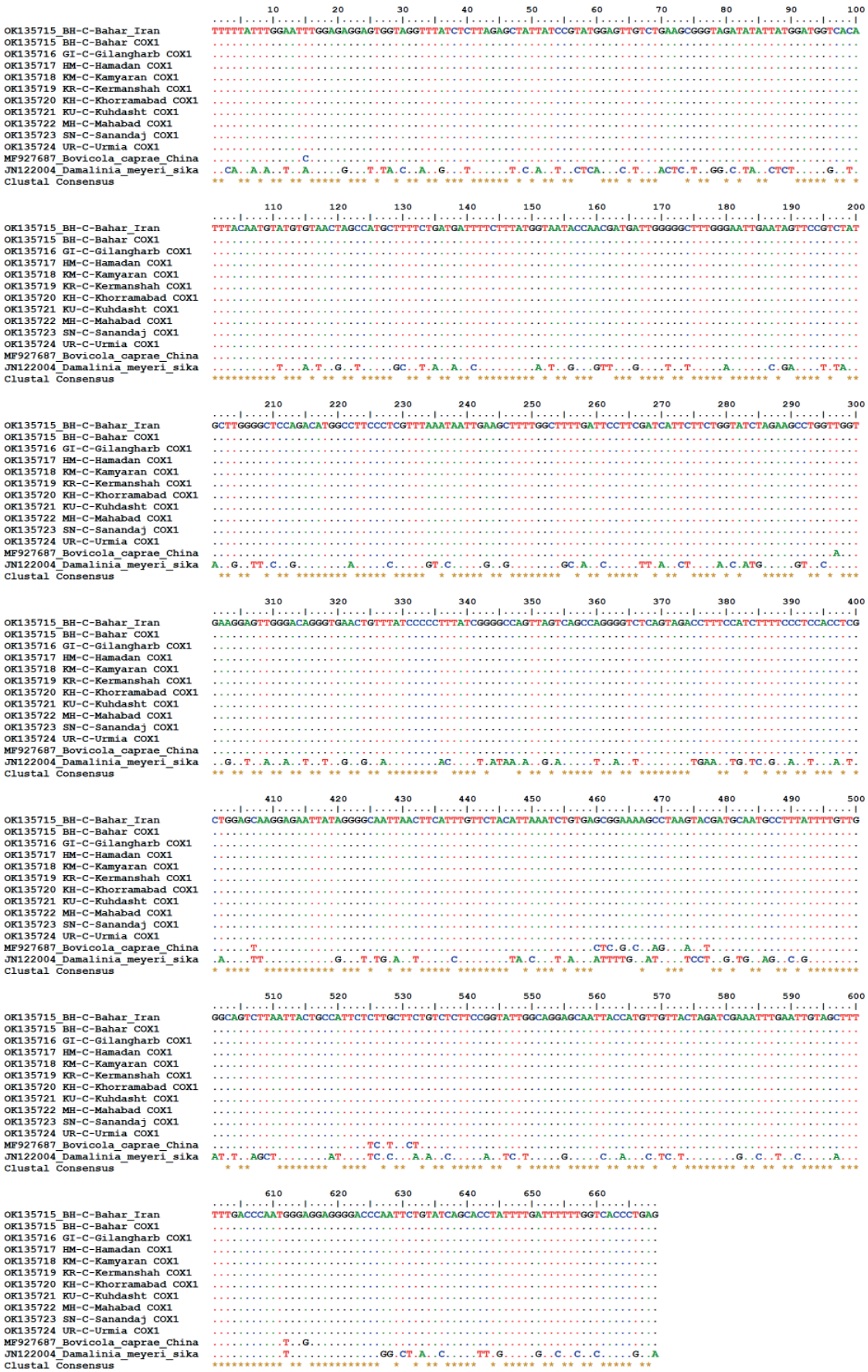

Figure 1.
Alignment results of all isolates in this study with the closest isolate in the GenBank and an outgroup sequence. The * sign in the last line of each row indicates the complete similarity in that row in terms of similar nucleotides.

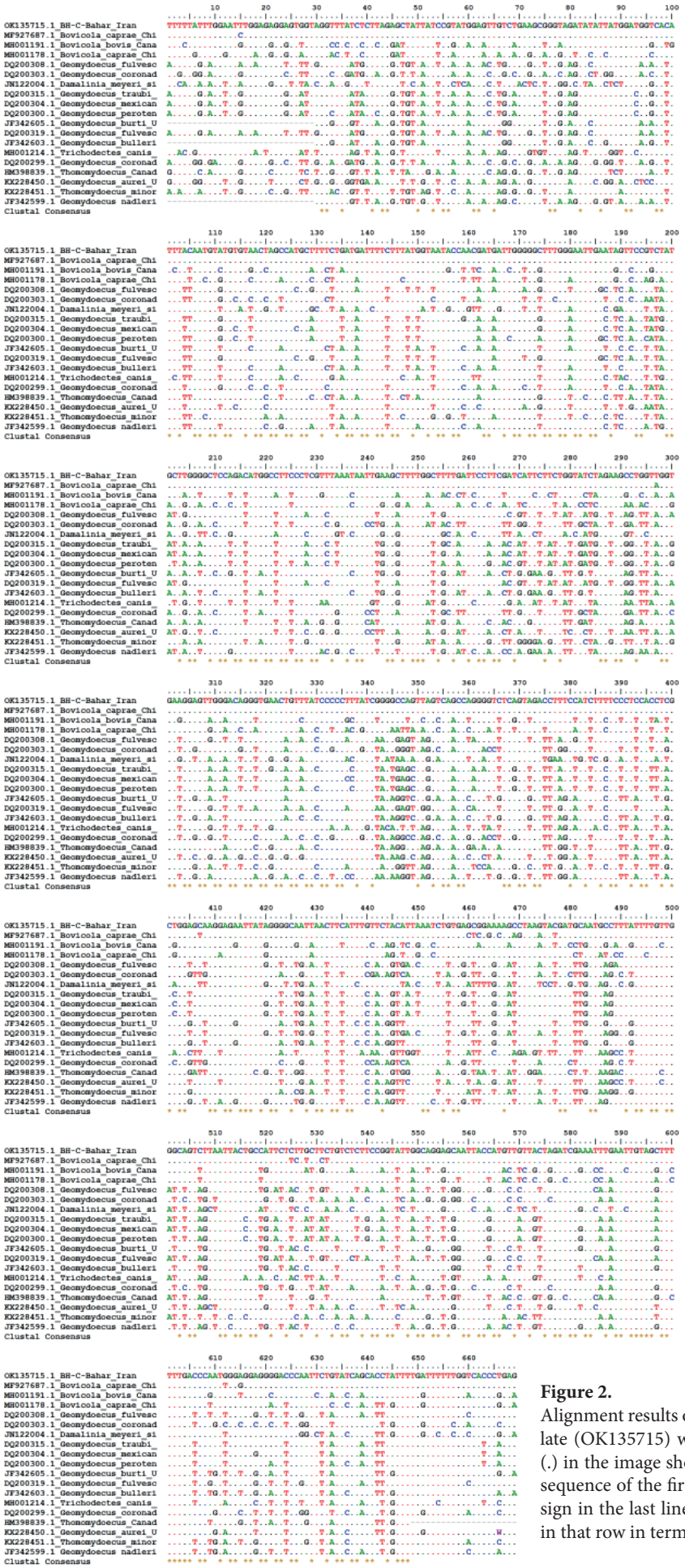

Figure 2.
Alignment results of nucleotide sequences of the city of Bahar isolate (OK135715) with sequences extracted from GenBank. Dots (.) in the image show the complete nucleotide similarity with the sequence of the first line related to the isolate of this study. The * sign in the last line of each row indicates the complete similarity in that row in terms of similar nucleotides.

Table 1.
Total number of *B. caprae* samples collected per city

Province	City	Latitude & Longitude	Species	Number of lice
Kermanshah	Kermanshah	34.1397° N, 45.9206° E	*Bovicola caprae*	122
	Gilangharb	34.3277° N, 47.0778° E	*Bovicola caprae*	130
Kurdistan	Sanandaj	35.3219° N, 46.9862° E	*Bovicola caprae*	71
	Kamyaran	34.7956° N, 46.9368° E	*Bovicola caprae*	86
West Azerbaijan	Urmia	37.5498° N, 45.0786° E	*Bovicola caprae*	102
	Mahabad	36.7684° N, 45.7337° E	*Bovicola caprae*	68
Hamedan	Hamedan	34.9083° N, 48.4393° E	*Bovicola caprae*	131
	Bahar	34.9083° N, 48.4393° E	*Bovicola caprae*	75
Lorestan	Khorramabad	33.4647° N, 48.3390° E	*Bovicola caprae*	83
	Kuhdasht	33.5275° N, 47.6111°E	*Bovicola caprae*	149

netic tree of the city of Bahar isolate (OK135715) and other sequences extracted from the GenBank showed that the isolate sequence of this study is next to the *B. caprae* sequence from China (MF927687.1) and *B. bovis* from Canada (MH001191.1). The closest resemblance was to the *B. caprae* sequence of China, shown in branch B1 (Figure 3). The phylogenetic tree of all isolates in this study along with the closest isolates in the GenBank showed a high similarity in terms of amino acid sequences. The analysis of one of the sequences related to this isolate (UAR89077.1 *B. caprae* Iran) was selected for comparison and bioinformatic studies. The alignment results of these amino acid sequences showed complete similarity to the sequences in this study (Figure 4). Drawing a phylogenetic tree based on the similarity of the amino acid sequence of the selected isolate (UAR89077.1 *B. caprae* Iran) and other amino acid sequences extracted from the GenBank showed that the isolate sequence of this study is mostly similar to the *B. caprae* sequence from China. Next to the AUV47083 sequence are *B. caprae* China and AYC65832 *B. bovis* Canada and AYC65818 *B. caprae* China, which are shown in branch B (Figure 5).

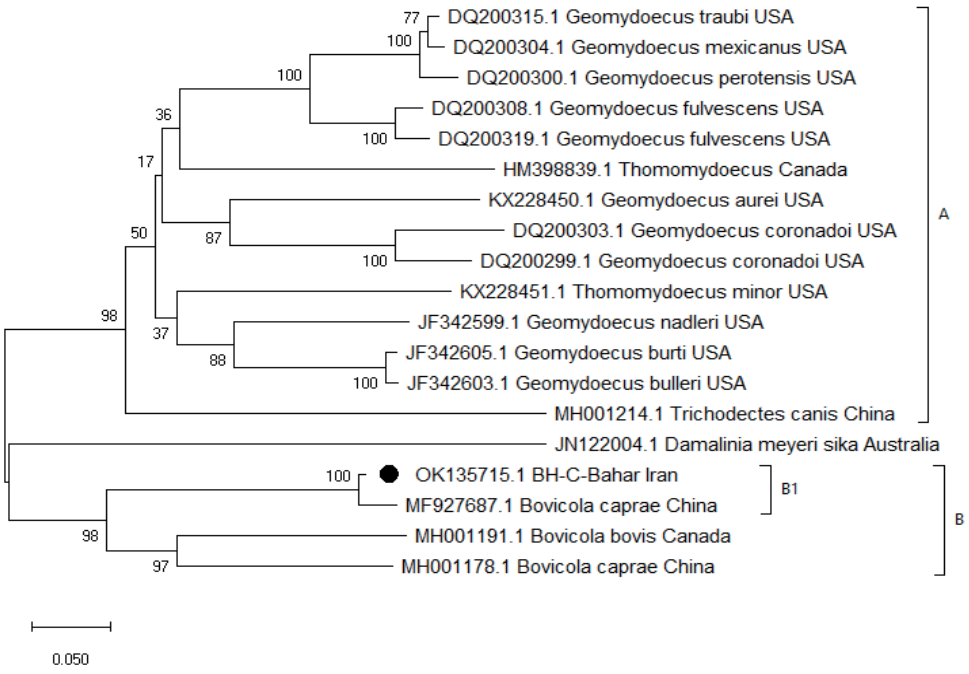

Figure 3.
Phylogenetic tree of Bahar isolate (OK135715) and other sequences extracted from the GenBank

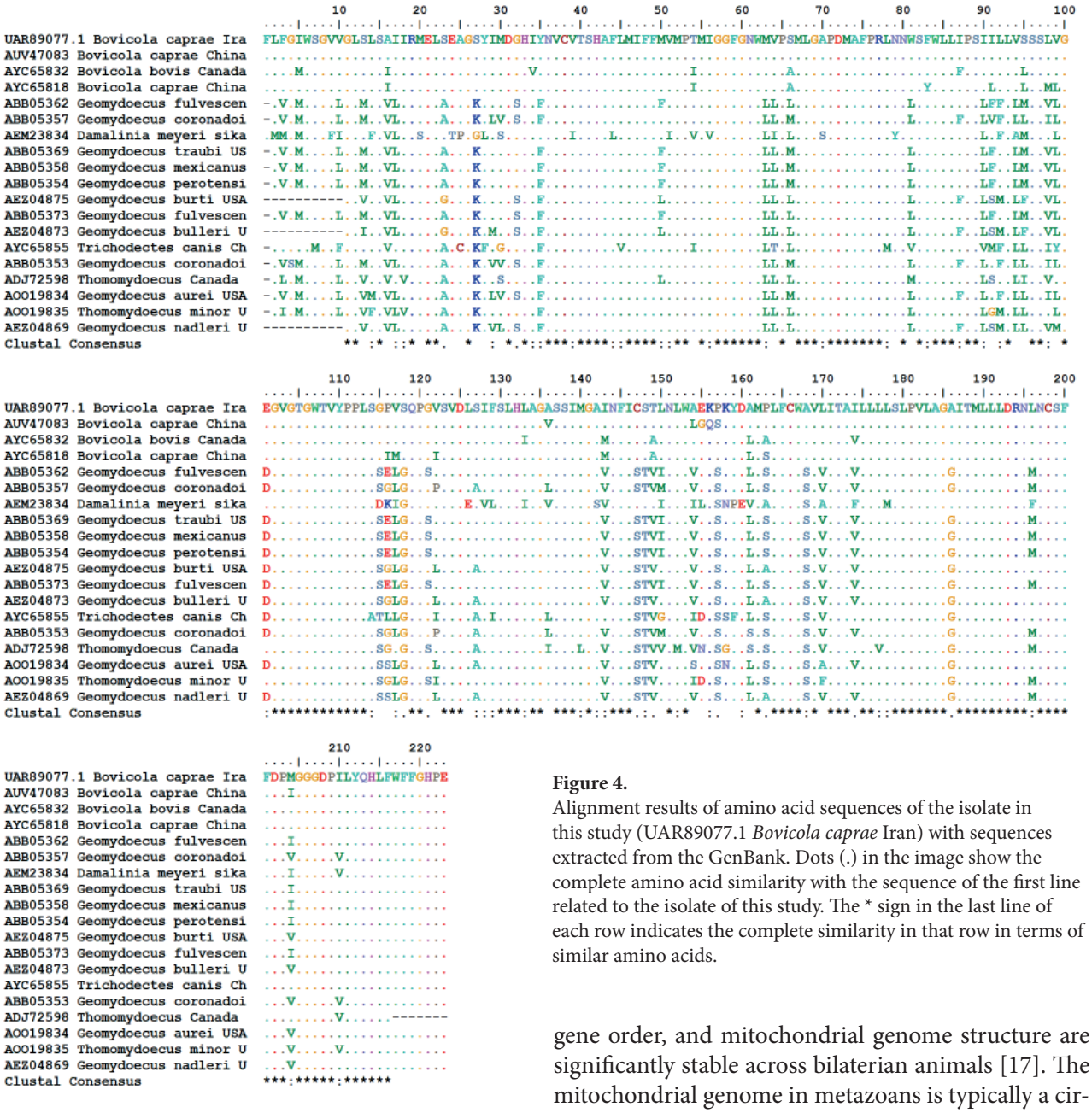

Figure 4.
Alignment results of amino acid sequences of the isolate in this study (UAR89077.1 *Bovicola caprae* Iran) with sequences extracted from the GenBank. Dots (.) in the image show the complete amino acid similarity with the sequence of the first line related to the isolate of this study. The * sign in the last line of each row indicates the complete similarity in that row in terms of similar amino acids.

Discussion

In this study, lice collected from goats in ten cities in the West and Northwest regions of Iran were studied morphologically and then phylogenetically. All collected specimens were identified as *B. caprae*. Yakhchali et al. (2006) investigated the ectoparasites of sheep and goats in Northwestern Iran and identified lice and ticks as the most common ectoparasites of small ruminants in this region and identified the species of *B. caprae* and *Linognathus stenopsis* in goats [15]. The species they identified in this region are consistent with the present research.

Many studies have revealed the value of the mitochondrial genome for the genetic study of population and intraspecific and systematic phylogeny in various organisms, including lice [16]. The gene composition, gene order, and mitochondrial genome structure are significantly stable across bilaterian animals [17]. The mitochondrial genome in metazoans is typically a circular DNA of 13-20 kb with 36-37 genes containing 12-13 protein-encoding genes, 2 rRNA genes, and 22 tRNA genes [16, 18]. However, the mitochondrial genome has an unusual structure in some species of lice and exists as small mini-chromosomes [16]. Among lice species, this fragmented structure was first found in the body lice *Pediculus humanus corporis*. The mitochondrial genome was then identified as small mini-chromosomes in some other species of lice, including *Bovicola caprae* [19-21]. The cox1 gene is a combination of highly conserved and variable regions that make this mitochondrial gene a molecular marker particularly useful for evolutionary studies [11]. The phylogeny of lice at the suborder level has not been resolved despite decades of study. Initially, all chewing lice from three suborders of Ischnocera, Amblycera, and Rhynchophthirina were collectively referred to as

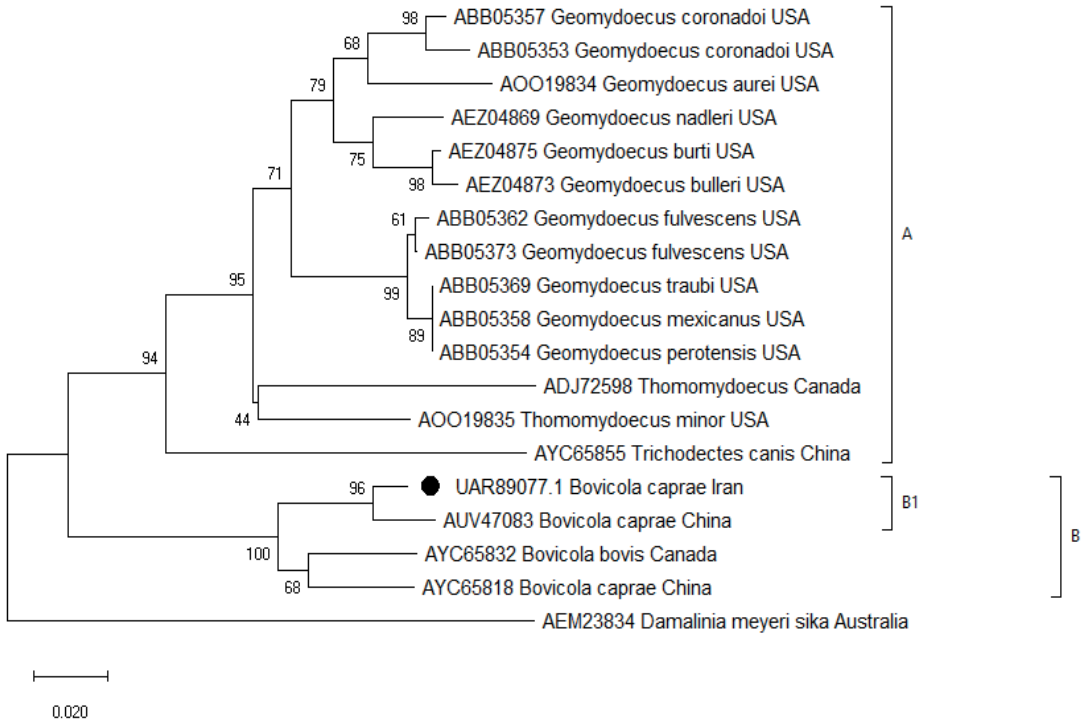

Figure 5.
Phylogenetic tree of the isolate in this study (UAR89077.1 *Bovicola caprae* Iran) and other amino acid sequences extracted from the GenBank

Mallophaga, but further studies showed that Mallophaga is paraphyletic [6]. Cruickshank et al. (2001) analyzed the EF1 sequence in 127 species from four suborders and reported that Ischnocera is paraphyletic [22]. Barker et al. (2003) analyzed 18SrRNA sequences from 33 species and reported Ischnocera as monophyletic [23]. Yoshizawa et al. (2010) analyzed five genes (*18S, histon3, wingless, rrnL, cox1*) and revealed Ischnocera as paraphyletic [24]. Recently, Johnson et al. (2018) analyzed nuclear genes of 46 species and reported Ischnocera as paraphyletic, and two species of Trichodectidae were grouped with the species of Anoplura and Rhynchophthirina [25]. Another study examined the nucleotide and amino acid sequence of protein-encoding genes *atp6, atp8, nad2, nad4, nad4l, nad6, cox1, cox2, cox3, cytb, rrnS, and rrnL* from three species of Hoplopleura. The *atp8* gene had the highest nucleotide diversity while the least diversity was observed in the cox1 gene. The latter finding is consistent with the present study [16]. Al-Shahrani et al. (2017) investigated phylogenetic differences based on two genes *cox1* and *cytb* in human head lice, and divided these lice into three classes [26]. Moreover, Mokhtar et al. (2019) examined the genetic diversity of human head lice using the genetic marker cox1 in Malaysia and reported that the collected samples belonged to clades A, B, and D [27].

This study is the first to demonstrate genetic variation in *B. caprae* lice collected from Western and Northwestern Iran using the cox1 marker. The results of morphological studies were consistent with molecular results and in general, 100% intraspecific similarity was observed in the nucleotide sequence of *B. caprae* samples isolated from ten cities based on the cox1 marker. Moreover, the alignment of the amino acid sequences also showed 100% similarity. *B. caprae* nucleic acid sequences in this study had 97.1% similarity to *B. caprae* (MF927687.1) from China and 77.1% similarity to *B. bovis* (MH001191.1) from Canada. *B. caprae* amino acid sequences in this study had 97.3% similarity to *B. caprae* (AUV47083) from China and 94.1% similarity to *B. bovis* (AYC65832) from Canada. The results of this study showed that cox1 is a useful marker to show intraspecific similarity. It is noteworthy that the cox1 gene sequence in this research contributed to the reference sequences available in the GenBank and also acts as a basis for a larger library of a goat chewing lice sequences in the West and Northwest of Iran.

The gene sequence of cox1 of the samples isolated in the present study is partial. Therefore, a comprehensive study is recommended to examine the complete sequences of this gene and compare and contrast comprehensive bioinformatic studies.

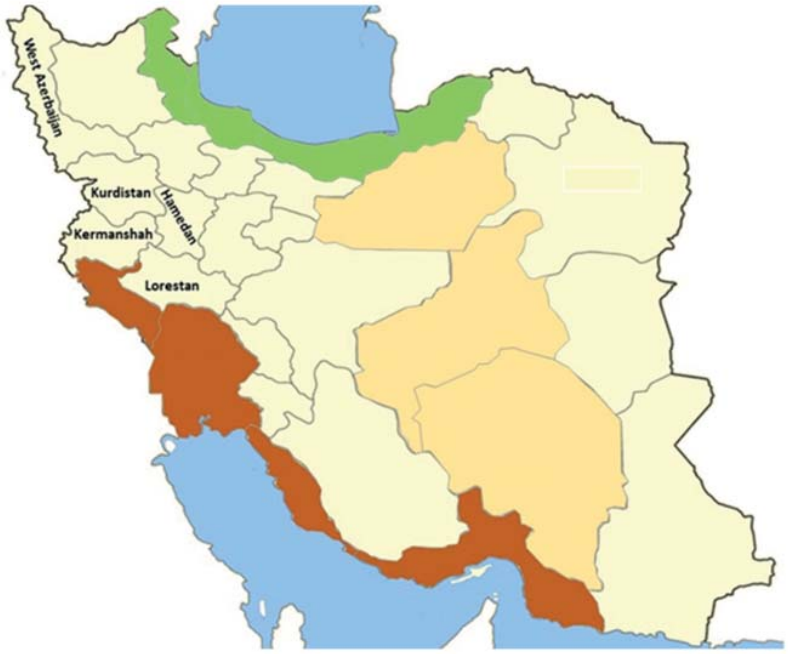

Figure 6.
The mentioned provinces in the current study

Materials and Methods

Sampling

This descriptive cross-sectional study was conducted during September 2017-March 2018 in five provinces in the West and Northwest of Iran (Figure 6). Samples were collected by direct sampling from goats' bodies in cities Urmia, Mahabad, Sanandaj, Kamyaran, Kermanshah, Gilangharb, Khorramabad, Kuhdasht, Bahar, and Hamedan. Out of 420 examined goats, 120 animals were infested by lice. Collected lice samples were fixed in 70% ethanol and transferred to the parasitology laboratory of Urmia Faculty of Veterinary Medicine and were identified using valid identification keys [7, 28, 29].

Morphological identification

All collected specimens of *B. caprae* were identified using morphological features as follows:

- Round head and antenna with three segments
- Distinct ocular points behind the antennae
- The ventral surface of the thorax with dark-colored plates
- All legs of similar size and with a single nail
- Cube abdomen with black side stripes
- Antennae in males are slightly larger than in females and have transverse bands
- Each abdominal segment has a middle spot and a row of short hair between the groove and the spots of each segment

DNA extraction and molecular diagnosis

Genomic DNA was extracted using a commercial DNA extraction kit of MBST (MBST, Tehran, Iran) according to the manufacturer's instructions. The DNA quality and concentration of each sample were evaluated using NanoDrop (Thermo Scientific 2000c, USA) by spectrophotometry and stored at -20°C until further evaluations. Primers designed by Folmer et al. (1994) as LCO1490 (5 - GGT CAA CAA ATC ATA AAG ATA TTG G-3) and HC02198 (5 -TAA ACT TCA GGG TGA CCA AAA AAT CA-3) were used to amplify a 669 bp fragment of cox1 [30]. PCR was performed in a volume of 50 µl and each microtube contained 5 µl DNA template, 5 µl 10X PCR buffer, 1 µl dNTPs (200 µM), 4 µl MgCl2 (50 mM), 1 µl of each primer (20 µM), and 1 µl Taq polymerase (Sinaclon, Iran). The PCR steps consisted of an initial DNA denaturation stage at 95°C for 5 min, and then 35 repetitions, each cycle involving denaturation at 95°C for 45 sec, primer annealing at 55°C for 45 sec, extension at 72°C for 45 sec, and a final extension step to complete polymerization at 72°C for 10 min. The PCR product was visualized using 1.5% agarose gel and UV-Transilluminator (BTS-20M, Japan). Finally, the PCR product was purified and sent along with forward and reverse primers to Takapouzist Co. (Tehran, Iran) for sequencing.

Sequencing and genomic analysis

Sequences were entered on the NCBI website to search for reference sequences with the highest similarity, and the BLAST method was used to find the positions of cox1. Afterwards, the sequences were aligned using MEGA software and any alignment error was resolved by the ClustalW method. All nucleotide sequences obtained in the GenBank were recorded with assigned access numbers, and phylogenetic relationships were investigated. To this end, a phylogenetic tree was drawn based on the maximum composite likelihood method with bootstrap test (1000 replicates) analysis

Authors' Contributions

Conceived and designed the experiments and revised the manuscript draft: KH.S., M.T. performed the experiments, analysed the data and drafted the manuscript: KH.S. All authors approved the final version of the manuscript.

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Competing Interests

The authors declare that there is no conflict of interest.

Reference

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Seasonal changes in serum progesterone levels in Caspian mares

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ABSTRACT

The present study was conducted to assess the seasonal breeding of Caspian horses in 10 mares during a year. Mares were divided into two age groups: 3-6 years (young) and 7-19 years (old). Blood samples (n=530) were collected weekly. The ovarian activity was evaluated by the concentration of progesterone. Mares with serum progesterone concentrations consistently higher and lower than 1 ng/ml were considered cyclic and non-cyclic, respectively. Results showed an interaction between time and age on the concentration of serum progesterone, ovarian activity, body weight, and body condition score (BCS) ($p < 0.05$). In March, the concentration of serum progesterone was higher in young mares (7.84 ± 1.14) than in old mares (1.26 ± 1.14, $p < 0.05$). The serum progesterone was higher in old mares than in young mares during July-November ($p < 0.05$). Ovarian activity was higher in young mares than in old mares during February-April ($p < 0.05$). Ovarian activity was higher in old mares than in young mares during July-November ($p < 0.05$). The length of the breeding season was higher in old mares than in young mares ($p < 0.05$). BCS was higher in young mares (4.4 ± 0.22) than in old mares (3.2 ± 0.22) in February ($p < 0.05$). Body weight was lowest in the young mares during September-January ($p < 0.05$). There was a significant correlation between ovarian activity and BCS of Caspian mares. Finally, seasonal breeding was shorter and earlier in young Caspian mares compared to old mares.

Keywords

Caspian horse, season, breeding, progesterone

Number of Figures: 3
Number of Tables: 4
Number of References:: 29
Number of Pages: 8

Abbreviations

BCS: Body condition score
ELISA: Enzyme-linked immunosorbent assay

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Introduction

The Caspian horse is intelligent and has great potential for training. This animal is very suitable for the equestrian education of children and adolescents due to its gentle behavior and body size. The Caspian horse is an ancient breed of small horse native to northern Iran (around the Caspian Sea) which is reported to be in danger of extinction in its original homeland [1]. Iranian Caspian horses were identified by Louise Firouz in 1966 and these animals were collected by Agricultural Research Education and Extension Organization (AREEO; Iran, Tehran) to be supported in 2008. A herd of 75 heads was provided, but over time, their number decreased. At present, this herd consists of 45 horses. It seems that less than 400 of these horses could be found in Iran [2]. Therefore, it is important to study the reproductive activity of this animal.

In seasonal breeding animals, the function of the reproductive system is adjusted to prevent the born of neonatal during bad weather. The mare is a seasonal polyestrous whose reproductive activity is induced by altering the photoperiod [3]. In addition to photoperiod, nutrition, body condition score (BCS) and environmental temperature affect seasonal reproductive activity in mare [4]. There is an interaction between these factors and photoperiod on the precise onset and duration of ovarian inactivity in the mare [5]. Moreover, the onset and termination of the breeding season may occur independently of a change in photoperiod [3].

Persistent ovarian activity is different in old and young mares [6]. Furthermore, the breed effect was reported for the time of the first ovulation and the end of winter ovarian inactivity in mares [7]. Evidence shows that the mortality rate is higher in foals born at the beginning of the year than in foals born later [8]. Different experimental approaches indicated that the annual reproductive rhythm of the mare, similar to other seasonal animal breeders, has a strong endogenous component [9]. Breeding information is available about some horse breeds. However, seasonal breeding information about Caspian horses is important and these data are needed to support this animal. Therefore, the present study aimed to determine the onset, termination, and length of seasonal breeding in Caspian mares.

Result

The results of analytical validation showed that the serial dilution of the serum Caspian mare was significantly decreased following the dilution level (r^2 = 0.9123, Figure 1). Moreover, the slope of the serial dilution of serum progesterone was also parallel to the

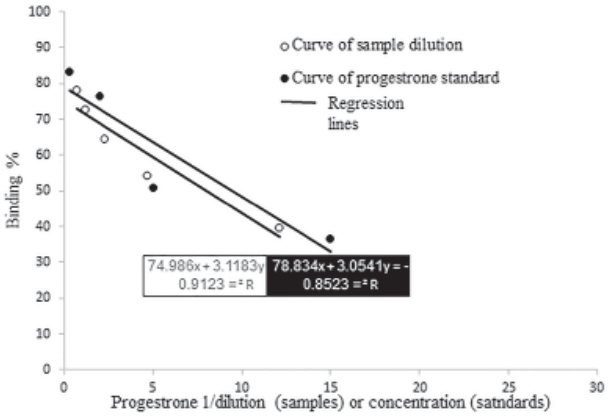

Figure 1.
Serial dilution results of Caspian mare serum are presented. The sample (open symbol) was diluted 1:2, 1:4, 1:8 and 1:16 in assay buffer and tested for binding to the progesterone conjugate antibody in parallel with serially diluted standard (closed symbol).

respective standard curves ($p < 0.05$). These results indicate that progesterone antigen in Caspian mare serum can bind correctly to the antibody of this assay. The scatter of serum progesterone concentrations in horses during the evaluation is shown in Figure 2. The serum progesterone concentration was higher in old mares than in young mares, (Table 1, p <0.05). The serum concentration of progesterone was higher in spring than in winter (p <0.05). There was an interaction between time and age on the serum concentrations of progesterone, ovarian activity, body weight and body condition score (BCS) (Figure 3, p <0.05).

There was no difference between mares on the serum progesterone from Dec to Feb (Figure 3A, p > 0.05). In March, the concentration of the serum progesterone was higher in the young mares (7.84 ± 1.14) than old mares (1.26 ± 1.14, p <0.05). There was no difference between the serum progesterone of old mares in April (6.36 ±1.14) and the serum progesterone of young mares in March (7.84 ± 1.01, p <0.05). In young mares, the serum progesterone was higher from March to July than that in other months (p <0.05). The serum progesterone of old mares was higher from April to November than that in other months (p <0.05). The serum progesterone was higher in old mares than that in young mares from July to November (p <0.05). In December and January, the minimal concentration of the serum progesterone was observed in all mares.

In young mares, the maximum (>80%) and minimum (<5%) ovary activity were observed from April to January (4 months) and from September to January (5 months), respectively (Figure 3B, p <0.05). In old mares, the maximum (>70%) and minimum (zero) ovary activity were observed from May to October (6 months) and from December to March (3 months),

Table 1.
The main effect of age and time on the serum progesterone, ovarian activity (monthly), body condition score and body weight of Caspian mares

Variable			Serum progesterone (ng/ml)	Ovarian activity (%)	Body condition score*	Weight (kg)
Age	Young		$3.88^{b} \pm 0.30$	$41.66^{b} \pm 4.53$	$4.40^{b} \pm 0.11$	$191.45^{b} \pm 3.59$
	old		$5.94^{a} \pm 0.30$	$53.41^{a} \pm 4.53$	$4.83^{a} \pm 0.11$	$218.99^{a} \pm 3.59$
Time	Winter	Jan	$0.08^{d} \pm 0.80$	$0.00^{f} \pm 7.4$	$3.20^{e} \pm 0.15$	$198.35^{d} \pm 2.72$
		Feb	$1.58^{d} \pm 0.71$	$14.00^{f} \pm 7.4$	$3.80^{e} \pm 0.15$	$201.20^{c} \pm 2.72$
		Mar	$4.55^{c} \pm 0.81$	$45.00^{e} \pm 7.4$	$4.6^{c} \pm 0.15$	$204.55^{c} \pm 2.72$
	Spring	Apr	$7.3^{b} \pm 0.71$	$80.00^{b} \pm 7.4$	$5.40^{b} \pm 0.15$	$208.50^{b} \pm 2.72$
		May	$9.85^{a} \pm 0.80$	$100.00^{a} \pm 7.4$	$5.90^{b} \pm 0.15$	$211.60^{a} \pm 2.72$
		Jun	$9.43^{a} \pm 0.71$	$98.00^{a} \pm 7.4$	$6.30^{a} \pm 0.15$	$213.80^{a} \pm 2.72$
	Summer	Jul	$9.30^{a} \pm 0.80$	$80.00^{b} \pm 7.4$	$5.50^{b} \pm 0.15$	$210.05^{ab} \pm 2.72$
		Aug	$7.20^{b} \pm 0.71$	$66.00^{c} \pm 7.4$	$5.20^{b} \pm 0.15$	$207.45^{b} \pm 2.72$
		Sep	$3.70^{cd} \pm 0.80$	$42.00^{d} \pm 7.4$	$4.20^{c} \pm 0.15$	$205.95b^{c} \pm 2.72$
	Fall	Oct	$4.04^{c} \pm 0.80$	$35.00^{e} \pm 7.4$	$4.10^{c} \pm 0.15$	$202.35^{c} \pm 2.72$
		Nov	$1.66^{d} \pm 0.80$	$10.00^{f} \pm 7.4$	$3.60^{e} \pm 0.15$	$200.50^{c} \pm 2.72$
		Dec	$0.13^{d} \pm 0.80$	$00.00^{f} \pm 7.4$	$3.20^{e} \pm 0.15$	$198.45^{d} \pm 2.72$

[a-g] different superscripts denote significant differences ($p < 0.05$)

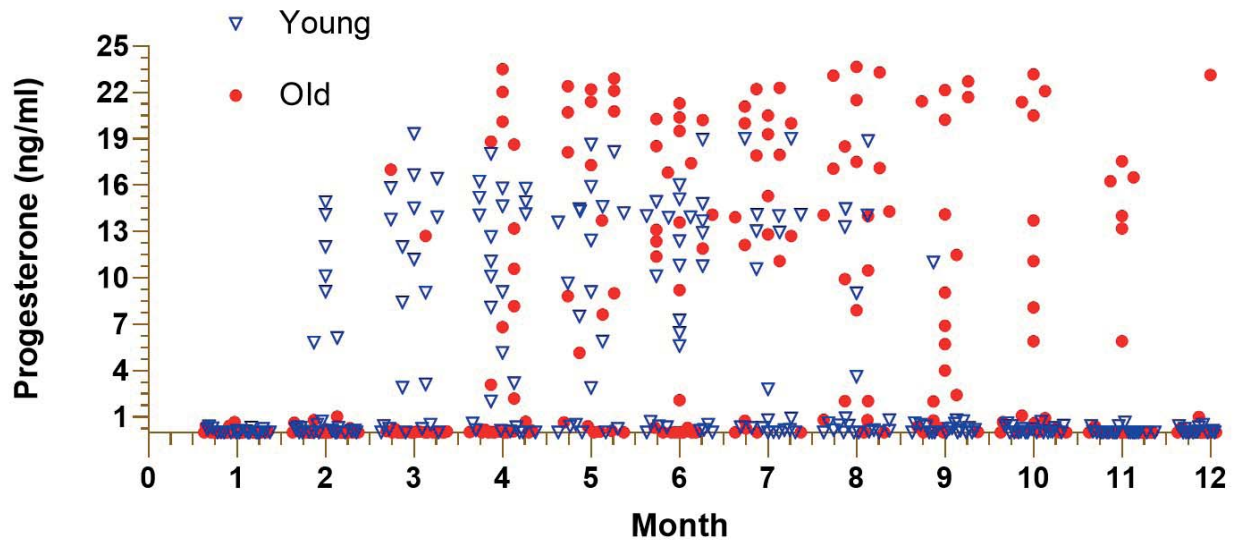

Figure 2.
Weekly changes in the serum progesterone concentration of each mare throughout the year

respectively (p <0.05). Ovary activity was higher in young mares than old mares from February to April (p <0.05). Ovary activity was higher in old mares than young mares from July to November (p <0.05). In May, ovary activity was highest in all mares (p <0.05). The length of the breeding season was higher in old mares than that in young mares (Table 2, p <0.05).

In December and January, the body condition score was minimum in all mares and there was no difference between mares (Figure 3C, $p > 0.05$). The body condition score was higher in young mares (4.4 ± 0.22) than that in old mares (3.2 ± 0.22) in February (p <0.05). In October and November, the body condition score was higher in old mares (p <0.05). Body weight was lower in the young mares (Figure 3D, p

<0.05). In January and July body weight was highest in old mares (p >0.05). Body weight was lowest in the young mares from September to January (p <0.05). There was a significant correlation between ovarian activity and body condition of Caspian mares (Table 3).

Table 2.
The length of the breeding season in Caspian horses

Age	The length of the breeding season (week)	From the winter solstice to the beginning of the breeding season (week)*	From summer revolution to the end of the breeding season (week)
Young	23.00^{b} ± 1.63	13.00^{b} ± 1.15	10.00^{b} ± 2.09
Old	27.80^{a} ± 1.63	19.40^{a} ± 1.15	21.40^{a} ±2.09

a-c different superscripts denote significant difference (p < 0.05)

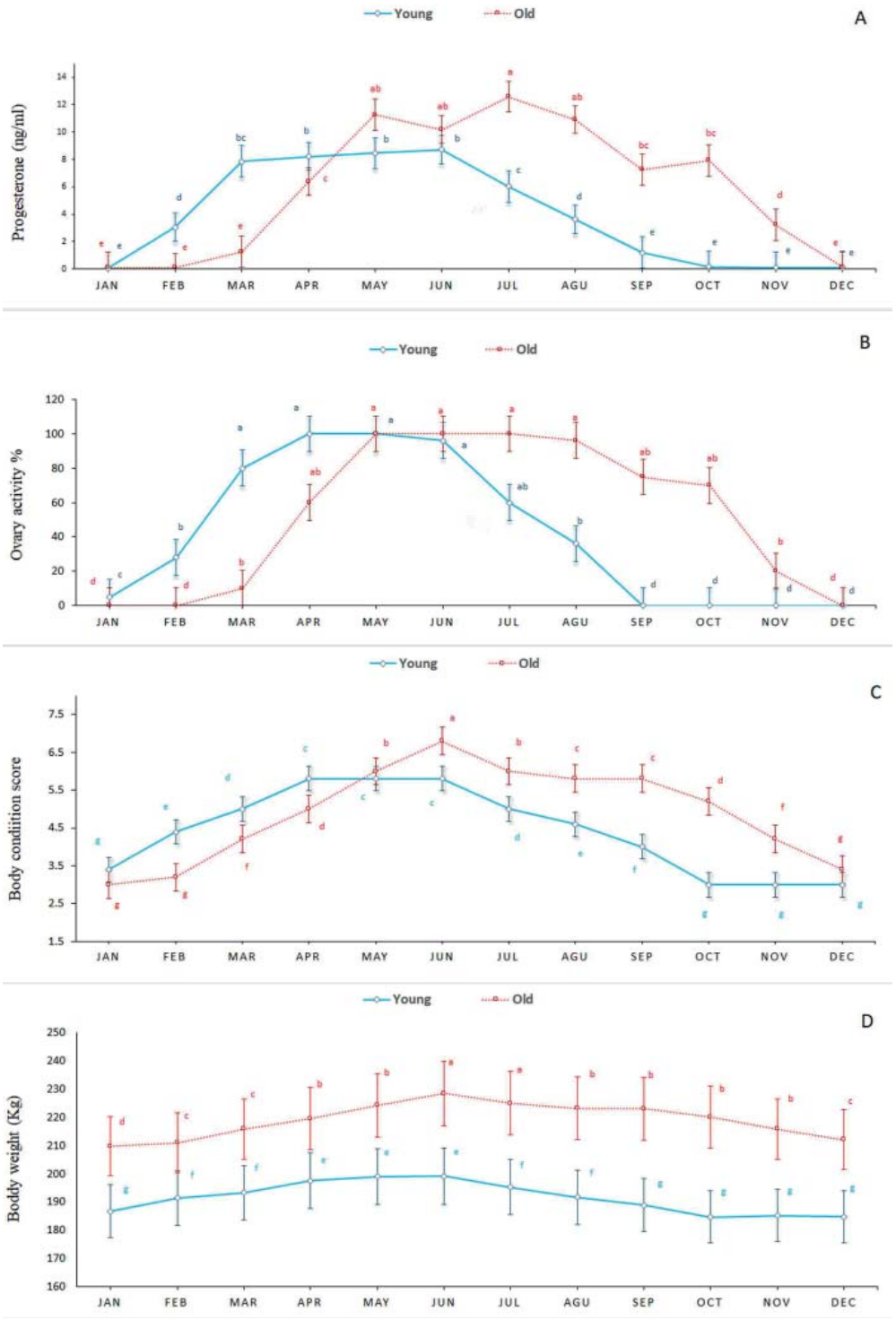

Figure 3.
The interaction effect of age and time on serum progesterone concentration (A), ovarian activity (B), BCS (C) and weight (D) in Caspian mares. a-g different superscripts denote significant differences (p < 0.05).

Table 3.
Correlation between ovarian activity, body condition and body weight in Caspian mares

Variable	Bodyweight (Kg)	Body condition	Ovarian activity
Ovarian activity	0.45256	0.91434	1.0000
	< 0.0001	< 0.0001	
Body condition	0.52675	1.0000	
	< 0.0001		
Body weight	1.0000		

Discussion

The results of the current research showed that the progesterone concentrations of young and old mares gradually began to increase at the beginning of the breeding season in February. Moreover, progesterone concentration was a lot higher in young mares than in old mares in March. Consequently, the spring transition in young mares started sooner than in old mares. In equine, a relative deficiency in the steroidogenic enzymes P450scc, P450c17, and P450arom was reported in spring transition [10]. It was mentioned that deficiency in steroidogenesis is due to the lack of gonadotrophic support caused by the low circulating concentrations of LH and low levels of gonadotropin receptors during the spring transition [11]. Supplementation of transitional mares with gonadotrophins in the form of hCG stimulates steroidogenesis [12]. Therefore, it seems that the ovarian steroidogenesis of young Caspian mares resumed earlier than old mares in the spring transition.

The BCS of young mares (> 4) was higher than old mares at the beginning of the breeding season (in February). Moreover, the spring transition and seasonal breeding of young mares began earlier than old mares. There was a significant correlation between ovarian activity and BCS, which is in line with the findings of Vecchi et al. [13]. Consequently, the onset of the breeding season might be modulated by the percentage of body fat and BCS in the Caspian mare. It has been mentioned that reproductive function is strongly influenced by metabolic hormones in mares [14]. Insulin can cross the blood-brain barrier and insulin receptors have been observed in several brain areas, including the hypothalamus [15]. Increased adiposity leads to a higher circulating concentration of insulin [16]. Moreover, hyperinsulinemia was observed in young mares in late winter [17]. Throughout the year, there is a correlation (0.64, $p < 0.001$) between insulin concentration and fat thickness in the mare [5]. Insulin modulates GnRH secretion [18, 19] and it seems to be also important for LH release [20]. However, in the equine species, this effect is controversial [21]. Findings suggest that the BCS and adiposity of Caspian mares are very important at the beginning of the breeding season.

This is the first study to report seasonal luteal activity in Caspian mares and a high percentage of horses with luteal activity in late spring and early summer. At the end of the breeding season, it was observed that the fall transition of young mares was during August-October. However, the fall transition of old mares was during late October-December. The breeding season of old mares started late but it took longer than young mares, which is similar to the results of other researchers [17, 22]. On the other hand, the BCS of old Caspian mares was > 4 from May to the end of November. It has been shown that the ovarian activity of the mares with high BCS continues during the winter conversely mares with BCS of < 3.5 [6]. During three years, BCS had a strong correlation with ovarian activity in Welsh ponies [5]. However, the ovarian activity of old Caspian mares, the same as the young ones, was completely consistent with the changes in the BCS throughout the year.

Our results showed differences between young and old mares regarding progesterone concentration, luteal activity, and BCS during the year. It was demonstrated that the concentration of serum leptin augmented with age and there was a positive correlation between BCS and serum leptin in horses, as leptin rose to 1.11 ± 0.57 ng/mL for one score increase in BCS [23]. Leptin, a hormone predominantly released by adipose cells, helps to regulate energy balance by inhibiting appetite [24]. On the other hand, low metabolic rates during winter (declining during autumn to reach a minimum during December and January) and the resumption of high metabolic activity in spring (augmenting exponentially during March and April to the annual peak in May) are well known in horses [25]. Therefore, the observed difference between young and old mares may have been due to seasonal changes in the metabolic rate and secretion of adipokines, such as leptin.

In conclusion, there were differences between young and old Caspian mares in the onset, termination, and length of the seasonal breeding of mare Caspian. Compared to old Caspian mares, the ovarian activity of young mares started and ended earlier. The length of the breeding season of young Caspian mares was shorter, whereas the ovarian activity of old Caspian mares continued until midwinter. There was a significant correlation between progesterone concentration and ovarian activity. Therefore, the differences

Haghdoost et al., IJVST 2022; Vol.14, No.3
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observed between the age groups of Caspian mares at the onset and end of the breeding season may result from the changes in BCS. It seems that age per se may not be an important variable.

Materials and Methods

Subjects and sample collection

The experiment was conducted on ten Caspian mares of the experimental herd from the Iran Meteorological Organization during June 2018-July 2019. Experimental procedures and protocols were performed under National Animal Ethics approved by the Veterinary Organization of Iran. Mares had not nursed a foal the previous year. These ten mares were randomly selected and divided into two age groups: 3-6 years (young) and 7-19 years (old). Mares had not nursed a foal the previous year. The horses were fed a diet formulated based on the National Research Council [25] feeding standard, body weight, age, and physical activity. The total daily dry matter intake of the horses was 2%-2.5% of body weight, with the same amount of forage and concentrate. The animals had free access to water and a salt mineral lick. The horses were given one-half of their daily diet of alfalfa hay and concentrate (Table 4) at 08:00 and 10:00, and the rest at 16:00 and 18:00, respectively. Housing and management conditions were the same for all animals. During the night, mares were housed in covered stalls and during the day they had access to a 300 m2 paddock.

Normal husbandry procedures, worm treatment, and vaccination programs were followed. All mares were kept under natural photoperiod, which at this latitude (37° 12′north latitude and 49° 39′east longitude) ranges from 8 h of light at the winter solstice to 16 h of light at the summer solstice. BCS and weight of mares were defined monthly [26, 27].

Blood samples

Since the beginning of the experiment, blood samples were collected by venipuncture of the jugular vein weekly. After clotting, sera were separated by centrifugation and were immediately used to determine progesterone concentration. The ovulatory activity was evaluated based on the concentration of progesterone.

ELISA

Serum progesterone was assessed in duplicate using a commercial ELISA kit (Monoblind, USA). A parallelism test was performed to define the reliably measure progesterone concentrations in the equine using a commercial ELISA. To examine the capability of commercial progesterone ELISA kits for measuring progesterone in Caspian horses, analytical validation was performed. The analytical validation comprises of parallelism test. Briefly, two sera from Caspian mares were diluted (1:2 to 1:16) using assay buffer. Diluted serum was then assayed together with progesterone standard (serial dilution of progesterone standard was 0.3-15 ng/ml). Afterward, the test of the equality of slope was performed following Zar [28] to compare the slope of the expected dose versus the percent bound of diluted serum with the slope of the standard dilutions.

Serum progesterone concentration

Mares were considered cyclic if serum progesterone concentration changed from < 1 ng/ml to > 1 ng/ml during 4 weeks. They were classified as being in anestrus if their progesterone levels were < 1 ng/ml for more than 4 consecutive weeks. The last date when the progesterone concentration was > 1 ng/ml in the last ovulatory cycle of the breeding season was deemed as the start of the anovulatory period, and the first date when progesterone was > 1 ng/ml in the first ovulatory cycle of the new breeding season was deemed as the end of the anovulatory period. Ovarian activity per month was calculated based on the following equation:

(Total number of samples per month-Number of samples (per month) indicated the anovulatory cycle)/(Total number of samples per month)×100

The relationships between ovarian activity, weight, BCS, and time were evaluated by the linear correlation analysis (CORR procedure) using SAS [29]. The data regarding serum progesterone concentration were analyzed by the MIXED procedure of SAS with repeated measures data, and least-squares means were compared using Tukey's adjusted method. The data on ovarian activity, weight, and BCS were analyzed by completely randomized design in a 3 (age groups) × 12 (months) factorial arrangement of 36 treatment combinations as fixed effects and time as a repeated measure. Mare was considered a subject in this experiment. The data on the length on the breeding season, the time interval between the winter solstice and the beginning of the breeding season, and the time interval between the summer solstice and the end of the breeding season were analyzed by a completely randomized design. Means were compared using Tukey's test and differences were considered to be statistically significant at $p < 0.05$.

Table 4.
Composition of feeds used for experimental mares.

Nutrients	Commercial concentrate	Alfalfa Hay
DE	3.4 Mcal/kg	2.43 Mcal/kg
CP	140 g/kg	150 g/kg
Ca	7.95 g/kg	14.7 g/kg
P	7.5 g/kg	2.8 g/kg
Mg	1.75 g/kg	2.9 g/kg

DE = Digestible Energy, CP = Crude Protein

Authors' Contributions

MRAM designed the experiment. RH and AG collected the samples. RH performed laboratory measurements. MRAM performed the analysis of data and wrote the manuscript. All authors contributed to data interpretation and revising the manuscript, as well as read and approved the final manuscript. MRAM had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Competing Interests

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

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Prevalence of *Melophagus ovinus* (Diptera, *Hippoboscidae*) in sheep in the province of Tungurahua, Ecuador

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ABSTRACT

Melophagus ovinus (Diptera: Hippoboscidae) is considered one of the main ectoparasites that attack sheep, however, in Ecuador information about this parasite is limited. In this study, the prevalence of *M. ovinus* in sheep was examined in Tungurahua province, Ecuador. For this purpose, sampling was performed in some semi-intensive and backyard sheep farming systems in different locations in Tungurahua. Significant differences were detected in the prevalence of *M. ovinus* between different locations, being higher in Ambato (39.3%), followed by Quero (33.5%), Mocha (32, 3%), Pelileo (30.7%), and Tisaleo (28%). Meanwhile, a significant decrease in prevalence was observed in Patate, Cevallos, and Píllaro ranging from 7.5% to 18%, and no *M. ovinus* was detected in Baños. No relationship was observed between the number of ectoparasites and the gender or age of the host sheep. A higher incidence was observed in males (58.45%) than in females (41.55%), even though it was not statistically significant according to Pearson's Chi-squared ($p = 0.492$). Similarly, when considering the effect of the age of an animal on the incidence of *M. ovinus*, no significant association was found based on Pearson's Chi-squared ($p = 0.314$). However, the animals aged 1-3 and older than 5 years showed a higher prevalence. This study highlights the need for further studies on the prevalence of *M. ovinus* in producing areas.

Keywords

sheep ked, ectoparasite prevalence, geographical distribution, Andean

Number of Figures: 3
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Abbreviations

M. ovinus: Melophagus ovinus
B. ovis: Bovicola ovis

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Introduction

The growth of the human population has brought about an increase in the demand for products obtained from livestock to satisfy the population's food needs. Therefore, the demand for sheep products has tended to increase since they are a source of meat, milk, and skin for a variety of uses [1]. Regarding sheep meat, worldwide production was 9,922,238 tons, of which 51.6% was produced in Asia, followed by Africa (20.8%), Oceania (11.9%), Europe (11.4%), and America with just 4.3% [2]. Among South American countries, Brazil, Argentina, and Peru contributed 38%, 20.8%, and 13.1% to meat production in this region, respectively, while in Ecuador, production was 5,838 tons representing 2.3% of the total amount produced in South America [2].

Despite the economical and nutritional importance of livestock for the Ecuadorian population, in 2019 there was a marked decrease in the number of cattle (6%), pigs (39.1%), and sheep (25%) in comparison with 2014 [3]. Some of the factors affecting livestock production were management issues, the genetic aspects of animals, and their interaction with the environment. It is well known that the climate affects animal welfare and production by influencing the quality and/or quantity of available food, the requirements of water and energy, and the amount of energy consumed [4]. Moreover, in recent years, climate change has made it necessary for both intensive and extensive production systems to promote strategies that allow adapting to environmental, social, and ecological changes and thus, moderate potential damages [5].

The prevalence of internal and external parasites is detrimental to livestock production, and ectoparasites, such as ticks, Hippoboscidae flies, lice, fleas, and scab mites can cause noticeable lesions on the coats of animals. They can also play a role in pathogen transmission and spoliation due to their blood-sucking habits [6]. According to Chilundo [7], although parasitic infections cause serious limitations to livestock production, these may be underestimated because they often do not cause clinical symptoms. However, they cause growth retardation and reduce fertility and productivity. According to Bedada et al. [8], ectoparasites are one of the main factors that affect sheep farming due to the economic losses they cause, mainly in small-scale production systems. In Ethiopia, these authors found a high prevalence of different species of ectoparasites, such as *B. ovis* (81.4%) and *M. ovinus* (19.2%), but the level of ectoparasite infestation had no relationship with age, gender, body condition, or management.

Similarly, Tamerat et al. [6] found 12 species of ectoparasites on sheep, with a higher prevalence of ticks (17.2%), followed by sarcoptiform mites (11.5%), lice (8%), and fleas (7.2%), mainly on animals showing poor body condition. According to these authors, the high prevalence of ectoparasites could negatively affect the production of small ruminants, which would then require the implementation of effective control measures to raise the production of these species. In addition to the damage to the skin or wool caused by various species of ectoparasites, they are also transmitters of pathogens. For example, *M. ovinus* is a transmitting agent of *Trypanosoma melophagium*, *Anaplasma ovis*, bluetongue virus, and various species of *Bartonella*, *Borrelia* spp., and *Rickettsia* spp., causing significant economic losses to sheep farming worldwide [9].

Considering the high incidence of ectoparasitic arthropods in sheep and their importance in the transmission of pathogens, in the present study, the prevalence of M. ovinus on sheep was investigated in Tungurahua province, Ecuador. Therefore, this study may constitute a baseline for future research that will focus on determining the possibility that these ectoparasite species are transmitting pathogens in the herds of the region.

Results

Morphological characterization of M. ovinus in sheep in Tungurahua province

All the collected specimens in the different sampling municipalities corresponded to *M. ovinus*, which presents a dorsoventrally flattened body, sunken head, thorax, and abdomen with the gnathal segments being of the prognathic type. Moreover, soft and flexible abdominal integument allows distension during feeding and larval development in females (Figure 1A). They are insects with small compound eyes, few ommatidia, and small immobile antennae located in deep antennal fossae. They are wingless because they are parasites that complete their entire life cycle on their host (Figure 1A).

In addition, they have robust legs with enlarged femurs, flattened tibiae, and short, compact tarsi with one or more basal teeth, which are shorter and more robust and with stronger tarsal claws in mammalian associated species, which allow them to cling to the skin or wool (Figure 1B). The morphological characteristics exhibited by the specimens collected in different sampling locations in the present study corresponded to those indicated by previous research. All members of Hippoboscoidea are morphologically adapted to an ectoparasitic life among the hair or feather of their hosts and, consequently, some parts of the body of these organisms have undergone modifications in response to permanent ectoparasitism [10].

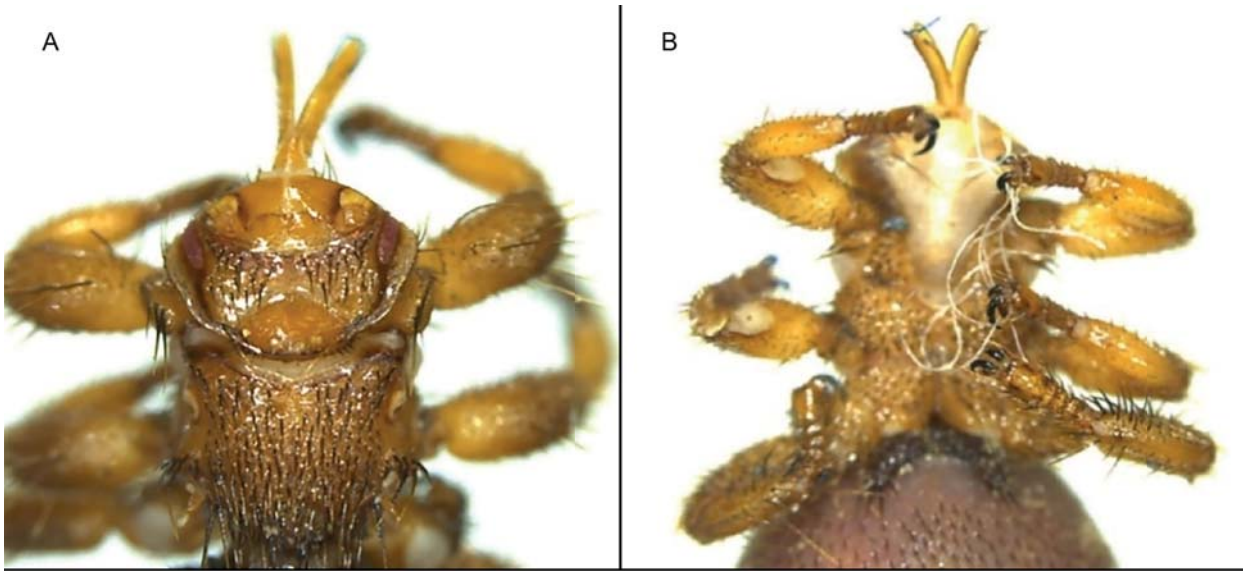

Figure 1.
Dorsal view of *M. ovinus* showing the short antennae within the antennal pit and the small compound eyes (A) and detail of the enlarged femurs, flat tibiae, and tarsi with strong nails (B).

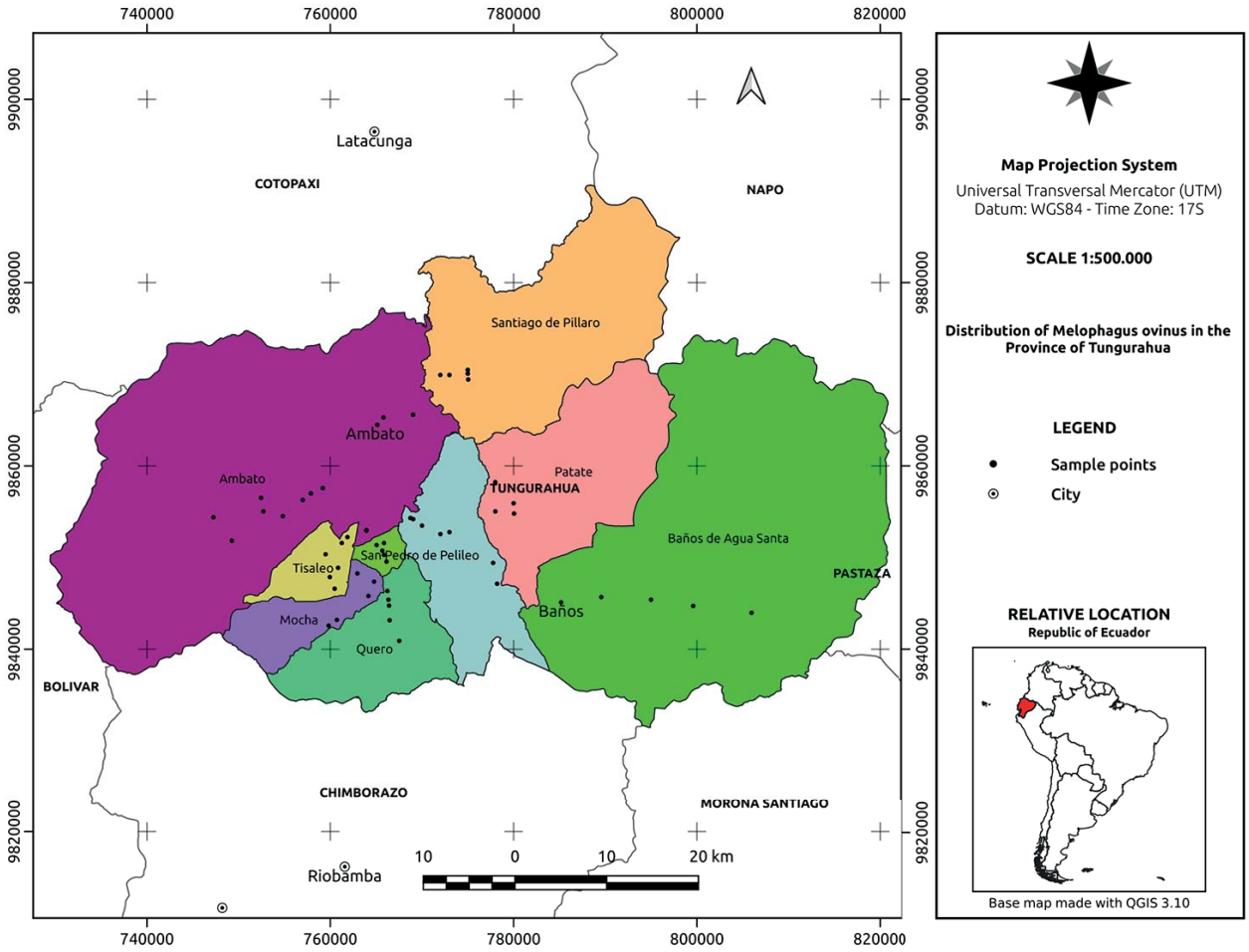

Figure 2.
Distribution map of *M. ovinus* in the different municipalities of the province of Tungurahua, Ecuador.

Hypoboscids are characterized by a dorsoventrally flattened body, a more or less flexible cuticle at the back of the body which allows the abdomen to expand after blood feeding or for keeping the larva during its development. The legs are strong with two claws to ensure the parasite has a good grip on the host, be it a bird or a mammal. In the case of M. ovinus, they have small compound eyes and antennae within an antenna cavity [11]. Hypoboscid ectoparasitism has led to the adaptation of some other structures, including prognathous buccal parts with two well-sclerotized palps called a proboscis that covers the stylets. This ends in a structure with sensillae and teeth to break the host's skin and feed on the blood. On the other hand, the legs have a pretarsus with a pair of claws, a pulvillus, and an empodium for the best hold [12].

Prevalence of M. ovinus in sheep in Tungurahua province

Significant differences were found in the prevalence of *M. ovinus* between different municipalities of Tungurahua province included in this study (Figure 3). In general, the highest prevalence was observed in the different localities of the Ambato municipality with a prevalence of 39.3%, which was statistically similar to that of the localities from Quero Municipality (33.5%), Mocha (32.3%), Pelileo (30.7%), and Tisaleo (28.0%). However, a lower prevalence was observed in Patate, Cevallos, and Píllaro, with a range of 7.5%-18%. It should be noted that no *M. ovinus* was detected in Baños. Furthermore, the greatest variation in prevalence was observed in Ambato, Cevallos, and Mocha, with the ranges of 10%-60%, 20%-50%, and 10%-50%, respectively. For the rest of the municipalities, the variation was lower (Table 1).

No relationship was found between the number of ectoparasites and the gender or age of the host sheep. A higher incidence was observed in males (58.45%) than in females (41.55%), which was not statistically significant according to Pearson's Chi-squared ($p = 0.492$). When the number of parasites by gender was analyzed, it was observed that a high percentage of animals presented 3-4 ectoparasites per animal in both genders (Table 2).

No significant association was observed between the animal's age and incidence of *M. ovinus* according to the Pearson Chi-squared ($p = 0.314$) (Table 3). However, a higher prevalence was revealed in animals aged 1-3 years and older than 5 years, with values of 31% and 29.6%, respectively, in which a high proportion presented 1-4 ectoparasites during sampling (Table 4). Based on the data in the localities considered in this study, the wide geographic distribution of *M. ovinus* in the municipalities of Tungurahua province is shown in Figure 2.

Discussion

Various studies have reported the prevalence of *M. ovinus* and other ectoparasites on sheep in Ethiopia. For example, the prevalence of *M. ovinus* reached 33.57%, which could be considered high due to its effects on animals' health and welfare [13]. This highlights the need to apply efficient sanitation practices in the stable and areas visited by the sheep for optimal control. In addition, they observed differences in prevalence due to the effect of the age and condition of animals with this being higher in young animals and animals with a poor body conditions. Although similar prevalence rates were found in the present study, no association was observed between the age of the animal and the prevalence of ectoparasite. Similarly, Mulugeta et al. [14] did not observe differences in the prevalence of *M. ovinus* and other species of ectoparasites concerning the age or gender of the host. On the other hand, they reported a higher prevalence of 52.4% in the highlands, while the prevalence decreased to 4.8% in the lowlands of medium height, and was not observed at all in low areas. This suggests that hot and humid climate conditions limit parasite distribution because temperature plays a key role in the distribution and population dynamics of *M. ovinus*. Moreover, these authors observed that *M. ovinus* was found more frequently in breeds with abundant wool, suggesting these breeds be more susceptible to parasite infestations.

Previous studies in different areas worldwide have demonstrated that temperature is considered the main environmental factor influencing *M. ovinus* distribution. However, even in regions with similar temperatures, the occurrence of this ectoparasite can vary, showing that apart from temperature, other environmental factors, such as thermal amplitude, humidity, and altitude, are likely to be involved [15]. In addition, there were other factors related to the host, including individual susceptibility and wool characteristics which could generate the necessary microclimate for parasite establishment. In this sense, the lack of correlation between similar temperatures and the abundance of *M. ovinus* in previous studies could explain the differences observed in the abundance of this species in the different municipalities of Tungurahua province with similar temperature regimes. Therefore, the observed variations could be attributed to other factors, such as herd management and differences in wool type due to the sheep breed. However, these factors need to be investigated to determine their role in the incidence of *M. ovinus* in the region.

Previous studies have shown that climatic conditions do not have a determining effect on the distribution, which further confirms this idea. Although *M. ovinus* was restricted to humid areas in the Pata-

Table 1.
Prevalence variation of *M. ovinus* in the sampled localities by municipality in Tungurahua province

Locality by municipality	Mean value ±S.D.	Maximum value	Minimum value
Ambato			
Atahualpa	9 ± 0.50	3	2
Chibuleo	28 ± 0.82	6	4
Comunidad San Isidro	10 ± 1.41	6	4
Echaleche	26 ± 0.84	6	4
Juan Benigno Vela	24 ± 0.63	5	3
Martínez	6 ± 0.00	2	2
Miñarica	17 ± 0.96	5	3
Montalvo	3 ± 0.71	2	1
Pasa	15 ± 1.71	6	2
Pilahuin	24 ± 0.84	6	4
Pisque	2 ± 0.00	2	2
Pucara Grande	5 ± 0.00	5	5
Santa Rosa	18 ± 0.89	5	3
Tamboloma	25 ± 1.47	6	2
Baños			
Lligua	0 ± 0.00	0	0
Rio Blanco	0 ± 0.00	0	0
Rio Negro	0 ± 0.00	0	0
Rio Verde	0 ± 0.00	0	0
Ulba	0 ± 0.00	0	0
Cevallos			
Agua Santa	2 ± 0.00	2	2
Andignato	6 ± 0.58	2	1
Ferrobiario	0 ± 0.00	0	0
La Florida	5 ± 1.15	3	1
Santa Rosa	6 ± 1.00	3	1
Mocha			
EL Rey	10 ± 1.15	4	2
Olalla	3 ± 0.00	3	3
Pinguili	10 ± 1.29	4	1
Primavera Alta	11 ± 1.53	5	2
Yanahurco	8 ± 1.41	5	3
Patate			
Bellavista	3 ± 0.71	2	1
Clementina	0 ± 0.00	0	0
Pelileo			
Benitez	9 ± 0.00	3	3
Chaupi	6 ± 0.00	3	3
Chilcapamba	6 ± 0.00	3	3
Pingue	6 ± 0.00	3	3
Salasaka	13 ± 0.50	4	3
San Jaloma Bajo	3 ± 0.00	3	3
Tambo	3 ± 0.00	3	3

Table 1 cont.

Pillaro					
Marco Espinel	4	±	0.58	2	1
Presidente Urbina	5	±	0.58	2	1
San Andrés	6	±	0.00	2	2
San José de Poaló	6	±	0.00	2	2
San Miguelito	6	±	0.00	2	2
Quero					
El Empalme	9	±	1.00	4	2
El Placer	11	±	0.58	4	3
Hualcanga Chico	11	±	0.58	4	3
Sabañag	14	±	1.29	5	2
Santuario	12	±	0.82	4	2
Tisaleo					
Alobamba	10	±	0.58	4	3
Barrio Olimpico	4	±	1.41	3	1
Bellavista	5	±	0.71	3	2
Quinchicoto Alto	6	±	0.00	3	3
San Diego	15	±	1.00	4	2
Santa Lucia	2	±	0.00	2	2

gonian region in Argentina, this species has recently spread to drier areas [16]. Concomitantly, studies on 123 sheep farms located in humid, mesic, and arid environments revealed that the prevalence of *M. ovinus* was higher than 72% [15].

Similarly, a study found differences in the prevalence of *M. ovinus* according to the location varying from 13.85% to 11.86% and 9.52% in Fura, Gorgo, and Dancye, respectively [17]. It was significantly lower in Wane (5.41%) and Wura (4.92%). Moreover, in Ethiopia, it was found that the prevalence of *M. ovinus* ranged from very low in Bahir-Dar at just 3% to a relatively high rate of 32.57% in Kombolcha, Ethiopia [18]. The differences were created not only by variations in climatic conditions but also by breeding practices, mainly related to the management of ecto-

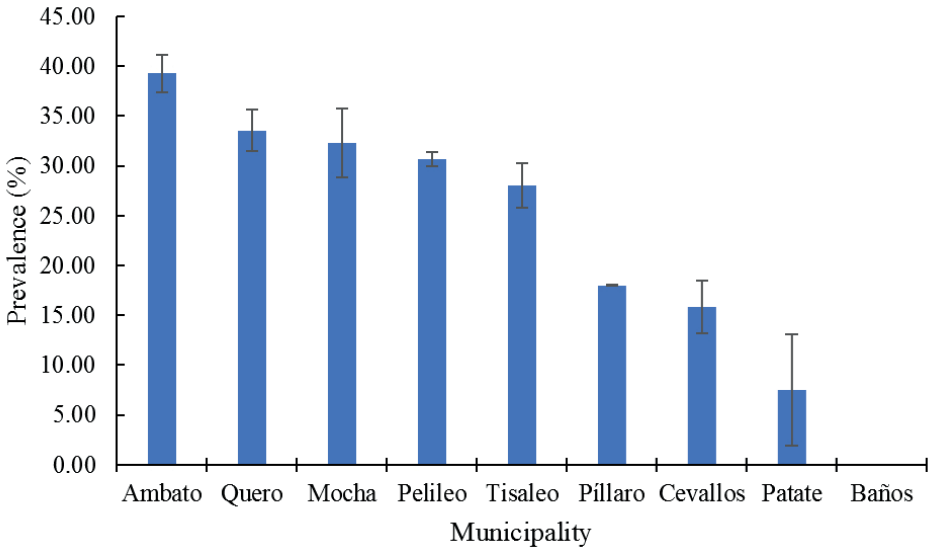

Figure 3.
Prevalence of *M. ovinus* in the different sampled municipalities of the province of Tungurahua

Table 2.
Relationship between the number of *M. ovinus* and sheep's gender

	Number of ectoparasites/animal				Pearson's *chi* square
	1-2	3-4	5-6	Total	
male	34	36	13	83 (58.45%)	
female	15	34	10	59 (41.55%)	0.492 n.s
total	49 (34.51%)	70 (49.30%)	23 (16.19%)	142 (100%)	

n.s.: not significant differences

Table 3.
Relationship between the number of *M. ovinus* and sheep's age

	Number of ectoparasites/animal				Pearson's *chi* square
	1-2	3-4	5-6	Total	
< 1 year	7	14	1	22 (15.50%)	*0.314n.s.*
1 – 3 years	19	19	6	44 (31.00%)	
3 – 5 years	11	16	7	34 (23.90%)	
> 5 años	12	21	9	42 (29.60%)	
Total	49 (34.50%)	70 (49.30%)	23 (16.20%)	142	

n.s.: not significant differences

Table 4.
Municipalities of Tungurahua province where the samples were taken

	Temperature (°C)	
	Maximum	**Maximum**
Baños	20	15
Cevallos	17	9
Mocha	15	7
Patate	20	13
Píllaro	17	10
Quero	16	9
Tisaleo	15	8
Ambato	20	9
Pelileo	20	8

parasites. These were influenced by the lack of knowledge among the farmers and the inadequate technical assistance provided by local governments [19].

The economic impact of *M. ovinus* and its potential to transmit pathogens highlight the need for more research on the prevalence of this ectoparasite in producing areas. Various studies have shown that apart from the physical damage and reduction in weight gain and reproduction caused by *M. ovinus* infestations, its ability to transmit different types of pathogens also highlights its economic importance for sheep farming at the national level and worldwide [20].

A series of pathogenic microorganisms can be transmitted by *M. ovinus*, including *Bartonella* spp., *Rickettsia* spp., *Candidatus Neoehrlichia mikurensis, Theileria* spp., which are the cause of different pathologies that compromise sheep productivity [21, 22]. In this case, it is suggested that the herd be regularly reviewed to detect excessive loss of fur, areas of skin irritation, and/or injuries that could be an indication of infestation with some type of ectoparasite, including, and thus, take prophylactic measures to reduce the possibility of infestation in the rest of the herd [23].

Materials and Methods

The prevalence of M. ovinus was estimated in sheep reared in semi-intensive and backyard systems. Random samplings were performed from April to July 2021 in different locations in the municipalities of Ambato, Baños, Cevallos, Mocha, Patate, Pelileo, Píllaro, Quero, and Tisaleo in the Tungurahua province, Ecuador (Table 4). Prevalence was expressed as the proportion of the population that showed positive results for *M. ovinus* at a given time [24], according to the following formula:

Prevalence=(Number of cases in the population at a given moment)/(Total population defined at the same moment in time)

Stratified sampling was used, which consisted of dividing the population into strata (based on age, gender, and condition of the animal) and samples were taken from each stratum [25]. The sample size was calculated using the formula given by Thrusfield [26] considering a precision level of 5% and a confidence interval of 95%. There are no previous studies on the prevalence of *M. ovinus* in the study area. Therefore, an expected prevalence of 50% of ectoparasites in small ruminants was assumed.

$$n = \frac{(1{,}96)^2 * P_{exp} * (1 - P_{\exp})}{d^2} = 384 \text{ animals}$$

Where "n" represents sample size, "P_{exp}" denotes Expected prevalence, and "d" is the expected absolute precision.

The animals under study were examined by the visual inspection of the back, folds, head, and neck to detect the presence of ectoparasites. Once the samples were obtained, they were placed in bottles with 70% alcohol until being processed in the laboratory. In addition to the samples, information on the gender and age (young, adult, and old) of the examined animals was recorded. In the laboratory, samples were assessed under a stereomicroscope to verify the morphological characteristics of the species according to Zhao et al. [27].

The obtained data were tabulated, including information on the sample number, sampling date, canton and sampling location, geographic coordinates, the number of parasites found, as well as the age and gender of the animal. Comparisons of prevalence between diverse localities, genders, and age groups were made using the statistical package Statistix version 10.0. In addition, photographs of the different regions of the body were taken. Finally,

a distribution map was prepared, each sampling site was georeferenced, and the geographic coordinate data were plotted using QGIS version 3.18.

Authors' Contributions

CV, ATB and SC conceived and planned the study. ATB carried out the samplings and contributed to sample preparation. CV, SC and GV contributed to the interpretation of the results and took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Competing Interests

The authors declare that there is no conflict of interest

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Effects of curcumin and its nano-micelle formulation on body weight, insulin resistance, adiponectin, and blood bio-chemical parameters of streptozotocin-induced diabetic rats

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ABSTRACT

The effects of curcumin and its nano-micelle form on body weight, insulin resistance, adiponectin, and blood bio-chemical parameters of streptozotocin-induced diabetic rats were studied. Diabetes was induced in fifty male Wistar rats which were divided into five groups treated with 1) no dietary supplements, 2 and 3) 40 and 80 mg curcumin/kg of feed, and 4 and 5) 40 and 80 mg nano-micelle curcumin/kg of feed. A group of ten untreated male Wistar rats was also considered a healthy control group. The serum concentrations of AST, ALT, glucose, insulin, triglycerides, cholesterol, HDL-C, LDL-C, and adiponectin, as well as insulin resistance, were assessed. Body weight and weight of liver, heart, and pancreas were also evaluated. Induction of diabetes increased the serum concentrations of AST, ALT, glucose, triglycerides, cholesterol, LDL-C, and insulin resistance and decreased the serum levels of insulin, adiponectin, and HDL-C, as well as body weight and weight of the heart and pancreas ($p < 0.05$). Nano-micelle form of curcumin alleviated the negative effects of glucose, lipid profile, and liver enzymes in diabetic rats ($p < 0.05$). In conclusion, the nano-micelle form of curcumin showed better efficiency compared to curcumin for improving the adverse effects of diabetes. It can be suggested that the nano-micelle form of curcumin at specific doses might be useful for diabetes treatment.

Keywords

curcumin, diabetes, hepatic enzymes, insulin resistance, nano-curcumin

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Abbreviations

AST: Aspartate aminotransferase
ALT: Alanine aminotransferase
HOMA-IR: Homeostatic model assessment of insulin resistance
HDL: High-density lipoprotein
LDL: Low-density lipoprotein
T2DM: Type 2 diabetes mellitus

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Introduction

Diabetes is a big challenge for most people in the world and T2DM is known for insulin resistance, faulted glucose, lipid metabolism, and deficient insulin production [1]. Therefore, increased levels of fasting glucose and postprandial glucose are its consequences [2]. Insulin resistance is the first sign of T2DM in most individuals. For maintaining normal glucose levels, beta cells raise insulin secretion and the response is observed as hyperinsulinemia. When hyperinsulinemia in patients cannot maintain normoglycemia, fasting blood glucose and glucose tolerance are faulted [3]. Faulted fasting blood glucose progresses to T2DM [4]. Several factors, such as glucotoxicity, lipotoxicity, inflammation, and accumulation of amyloid disturb beta-cell function [2]. Problematic lipid and glucose metabolism promote the pathogenesis of T2DM [4]. Adiponectin, an insulin-sensitizing hormone with anti-apoptotic and anti-inflammatory effects, is produced in adipose tissues and its levels decline in patients with T2DM [5]. The serum concentrations of AST and ALT increase in diabetes [6]. Augmented levels of these enzymes show hepatic injury in both the hepatocellular cytosol and mitochondria [7]. Increased ALT and AST are strongly correlated with insulin resistance and T2DM [8].

The control and management of diabetes are challenges for the medical system. Different manipulations are used for the treatment of diabetes, such as anti-diabetic medications and lifestyle intervention [9] (healthy nutrition and daily physical activity). The use of synthetic compounds may induce severe side effects, including hypoglycemic coma and hepatorenal disorders [9]. Medicinal plant supplements are recommended with high potency for preventing and managing T2DM [10-13]. Curcumin is an active molecule in the rhizome of turmeric. It has antioxidant, anti-inflammatory, anti-microbial, immunomodulatory, hypoglycaemic, and anti-rheumatic effects [14, 15]. Curcumin controls glycemia and lipidaemia in the body [16] and can be used as an appropriate compound for diabetes treatment. The use of natural isolates from plants is an appropriate strategy for the treatment of different disorders. However, curcumin users face major limitations due to formulation, application, and degradation during processing [17, 18]. Using the nano-micelle form of curcumin may prevent its degradation during processing and digestion and helps to increase its efficiency. Therefore, this study was conducted to investigate the effects of curcumin and its nano-micelle form on body weight, insulin resistance, adiponectin secretion, and blood biochemical parameters of streptozotocin-induced diabetic rats.

Results

The effects of treatments on the serum concentrations of glucose, insulin, adiponectin, and insulin resistance of the rats are shown in Figure 1. The results showed that diabetes induction increased the serum concentration of glucose ($p < 0.0001$) and decreased the serum levels of insulin ($p < 0.0001$), HOMA-IR ($p < 0.0001$), and adiponectin ($p < 0.0001$). Our findings indicated that the administration of curcumin at the level of 40 mg/kg of rat diet and nano-micelle curcumin in both doses reduced the serum concentrations of glucose ($p < 0.05$) and raised insulin ($p < 0.05$), HOMA-IR ($p < 0.05$), and adiponectin ($p < 0.05$). The best responses were observed in nano-micelle curcumin groups, especially in the rats which received 80 mg/kg of diet ($p < 0.05$).

The effects of treatments on the serum lipid profile of the rats are presented in Table 1. The results of the present study revealed that the induction of diabetes significantly increased the serum concentrations of triglycerides ($p < 0.0001$), cholesterol ($p < 0.0001$), and LDL-C ($p < 0.0001$) and diminished HDL-C ($p < 0.0001$). Administration of curcumin had no significant effect compared to the control group ($p > 0.05$). The administration of nano-micelle curcumin decreased the serum levels of triglycerides ($p < 0.0001$), cholesterol ($p < 0.0001$), and LDL-C ($p < 0.0001$) and increased HDL-C ($p < 0.0001$). No significant difference was observed between the nano-treatments ($p > 0.05$).

The effects of treatments on body weight and relative percentages of pancreas, heart, and liver of the diabetic rats are shown in Table 2. Our results showed that diabetes induction reduced body weight ($p < 0.0001$) and the relative weight of pancreas ($p < 0.05$) and heart ($p < 0.05$), but did not have any significant effect on the liver weight ($p > 0.05$). The results did not show any significant differences between diabetic treatments for the relative weight of the pancreas and heart. Supplementing 40 mg curcumin/kg of feed decreased body weight compared to the diabetic control group ($p < 0.05$).

The effects of treatments on serum levels of the liver enzymes of diabetic rats are shown in Table 3. Induction of diabetes raised the serum concentrations of ALT and AST ($p < 0.0001$). The current research demonstrated that nano-curcumin administration at both doses decreased the serum concentrations of ALT and AST ($p < 0.0001$). Serum ALT was not affected by 40 and 80 mg curcumin/kg of feed ($p > 0.05$).

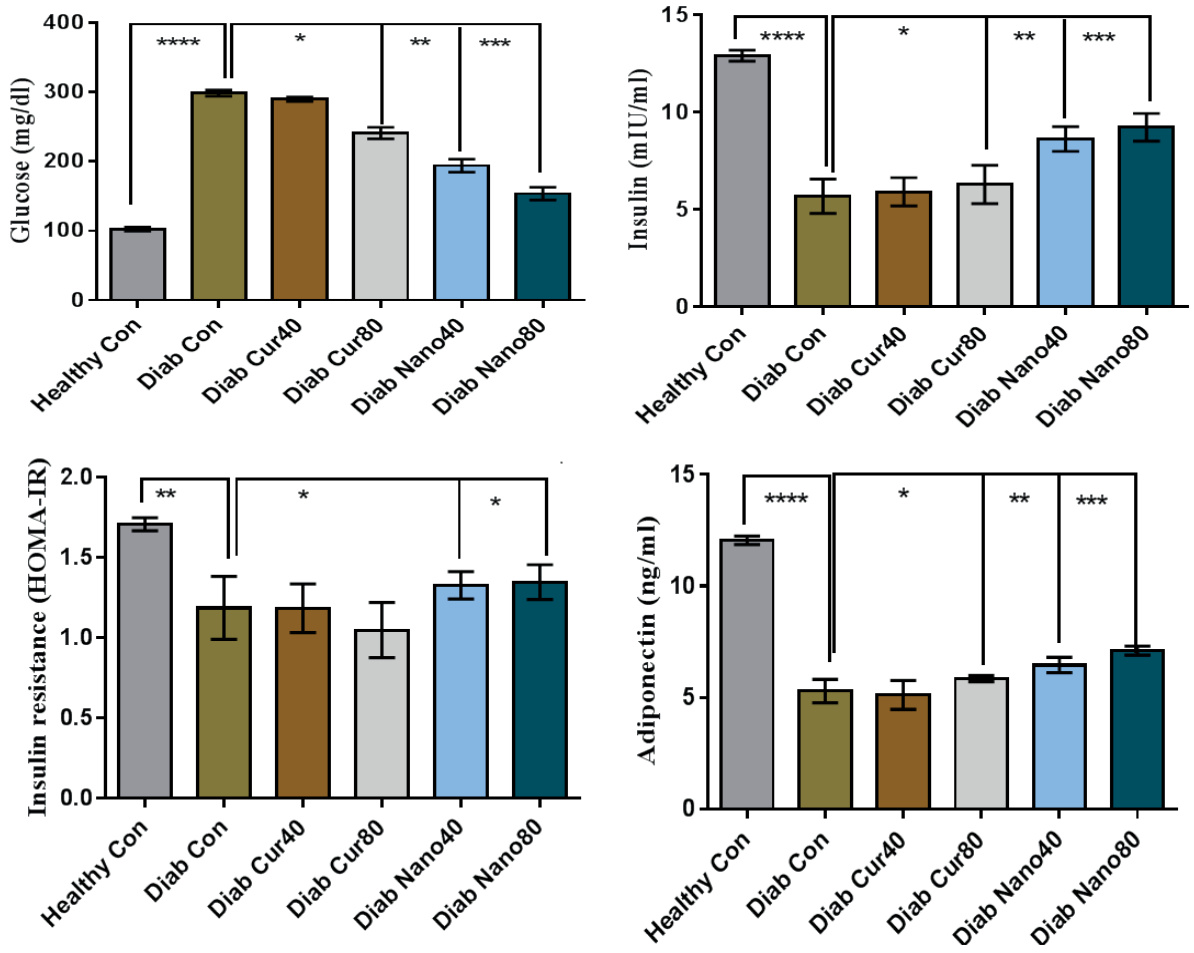

Figure 1.
Effects of experimental treatments on the serum concentrations of glucose, insulin, adiponectin, and insulin resistance of the diabetic rats. *, **, ***, and **** show significant differences at 0.05, 0.01, 0.001, and 0.0001, respectively. Cur: Curcumin; Nano: nano-curcumin; Healthy Con: Healthy control rats; Diab Con: Diabetic Control rats with no dietary supplement; Diab Cur40, Diab Cur80, Diab Nano40, and Diab Nano80 are 40 and 80 mg curcumin and nano-micelle curcumin/kg of feed, respectively.
HOMA-IR: Homeostatic model assessment of insulin resistance index was measured based on the product of fasting serum glucose concentration (mmol/l) and fasting blood serum insulin concentration (μU/ml) divided by the constant 5.22×18 as reported by Matthews et al. [20].

Table 1.
Effects of treatments on the serum lipid profile (mg/dl) of the diabetic rats

	Triglycerides	Cholesterol	HDL-C	LDL-C
Healthy Control	50.20 ± 5.49^{c}	116.60 ± 4.21^{c}	52.80 ± 3.96^{a}	53.76 ± 7.08^{c}
Diabetic Control	89.60 ± 3.64^{a}	185.60 ± 8.56^{a}	26.75 ± 1.50^{c}	142.10 ± 8.41^{a}
Diabetic Cur40	89.00 ± 3.74^{a}	182.40 ± 2.51^{a}	26.20 ± 2.28^{c}	138.40 ± 4.36^{a}
Diabetic Cur80	88.60 ± 3.78^{a}	179.80 ± 2.58^{a}	28.60 ± 2.60^{c}	133.50 ± 4.50^{a}
Diabetic Nano40	82.80 ± 3.56^{b}	171.60 ± 2.07^{b}	32.75 ± 2.07^{b}	122.20 ± 2.12^{b}
Diabetic Nano80	81.20 ± 2.77^{b}	166.40 ± 2.96^{b}	34.20 ± 2.68^{b}	116.00 ± 3.90^{b}
P-value	0.000	0.000	0.000	0.000
SEM	**2.640**	**4.420**	**1.850**	**5.960**

$^{a-c}$ Means in each column with different superscripts are significantly different ($p < 0.05$). SEM: Standard error of means. Curcumin (Cur) and nano-micelle curcumin (Nano) with specified doses of 40 and 80 mg/kg diet. HDL-C, high density lipoprotein-cholesterol, LDL-C, low density lipoprotein-cholesterol.

Table 2.
Effects of treatments on body weight (g) and the relative weights (w/w*100) of the pancreas, heart, and liver of the diabetic rats

	Body weight	Pancreas	Heart	Liver
Healthy Control	308.80 ± 16.65[a]	0.51 ± 0.35[a]	1.18 ± 0.04[a]	10.04 ± 0.45
Diabetic Control	224.60 ± 3.71[b]	0.23 ± 0.02[b]	0.98 ± 0.03[b]	10.22 ± 1.16
Diabetic Cur40	191.00 ± 27.48[c]	0.36 ± 0.06[ab]	0.90 ± 0.13[b]	10.37 ± 2.50
Diabetic Cur80	243.40 ± 53.90[b]	0.40 ± 0.19[ab]	0.89 ± 0.10[b]	10.59 ± 1.13
Diabetic Nano40	209.00 ± 15.17[bc]	0.35 ± 0.04[b]	0.87 ± 0.06[b]	11.13 ± 0.90
Diabetic Nano80	225.00 ± 15.00[bc]	0.42 ± 0.09[ab]	0.85 ± 0.03[b]	10.34 ± 1.07
P-value	0.000	0.013	0.000	0.848
SEM	**8.250**	**0.023**	**0.024**	**0.023**

[a-c] Means in each column with different superscripts are significantly different ($p < 0.05$). SEM: Standard error of means. Curcumin (Cur) and nano-micelle curcumin (Nano) with specified doses of 40 and 80 mg/kg diet.

Discussion

The results of the present study showed that the induction of diabetes increased the level of glucose and decreased insulin. The latter findings have already been reported by others [1-3]. Diabetes destroys beta-cells and hereby increases blood glucose levels and decreases insulin concentrations. Our results revealed that the oral administration of 80 mg curcumin and nano-curcumin/kg of feed reduced the glucose level and raised serum insulin concentration. The difference between curcumin 40 and control diabetic groups was not significant. It means that nano-curcumin can alleviate the adverse effects of diabetes on glucose and insulin levels. Curcumin is known to have an anti-hyperglycaemic effect in diabetic subjects [21-23]. Previous studies indicated that the administration of curcumin improved insulin sensitivity by reducing glycemia and dyslipidemia in rats when they are fed a high-fat diet [24-26]. In agreement with the results of the current study, Lu et al. [27] showed that curcumin supplementation improved glucose and insulin intolerance by activating the 5'-adenosine monophosphate-activated protein kinase pathway in diabetic animals. Administration of nano-micelle curcumin showed a better response compared to curcumin. Low absorption rate and rapid degradation of curcumin in the intestinal system have been reported [28]. utilizing the nano-micelle structure of curcumin prevents rapid degradation and promotes absorption in the intestinal system, leading to better anti-hyperglycaemic function. Diabetes may destroy pancreas function and insulin secretion because it disturbs the oxidant-antioxidant balance. It seems that using the nano-micelle form of curcumin increases antioxidant properties and prevents pancreas injury. Therefore, the administration of nano-micelle curcumin decreases the level of glucose and subsequently reduces the serum concentration of insulin. As observed in the current study, insulin resistance improves following improved insulin concentration and pancreas injury.

Table 3.
Effects of treatments on the serum levels of the liver enzymes of the diabetic rats

Treatments	ALT	AST
Healthy Control	22.11±0.84c	320.90±0.97e
Diabetic Control	37.58±0.49a	381.60±5.90a
Diabetic Cur40	36.62±0.49a	370.60±5.39b
Diabetic Cur80	36.02±0.44a	363.80±2.55b
Diabetic Nano40	35.36±0.83b	350.20±4.71c
Diabetic Nano80	34.64±1.14b	334.60±1.14d
p-value	0.000	0.000
SEM	0.980	3.930

[a-e] Means in each column with different superscripts are significantly different ($p < 0.05$). SEM: Standard error of means. Curcumin (Cur) and nano-micelle curcumin (Nano) with specified doses of 40 and 80 mg/kg diet. ALT: alanine aminotransferase; AST: aspartate aminotransferase.

The results showed that diabetes decreased adiponectin. However, curcumin administration at a higher dose and nano-micelle form as 40 and 80 mg/kg of feed raised the level of adiponectin, and the best response was observed in the rats fed nano-micelle curcumin. Adiponectin is produced in the adipose tissue, and its tissue levels directly correlate with its func-

tion. Adiponectin is an insulin-sensitizing hormone with anti-apoptotic and anti-inflammatory effects. Adiponectin declines in the adipose tissues of patients with T2DM [5]. Curcumin corrects the improper function of the nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-kB) in adipocytes and may improve adiponectin levels [29]. NF-kB links to cancer, inflammatory conditions, autoimmune diseases, and improper immune development. It controls the transcription of DNA, cytokine production, and cell survival. It is well-known that NF-kB regulates inflammatory responses [30]. Our findings showed a direct relationship between insulin and adiponectin. It means that the administration of curcumin increases insulin levels and subsequently augmented adiponectin levels. Diabetes increased the concentration of triglycerides, cholesterol, and LDL-C, and decreased HDL-C. Exclusively the administration of nano-curcumin could improve the serum lipid profile of rats. Diabetes disturbs lipid metabolism [1] and nano-curcumin improved blood lipid profile in diabetic rats compared to the control group. These results are consistent with the previous findings [29, 31]. Improved lipid profile could be attributed to the rise in lipoprotein lipase activity that reduces serum triglycerides. Moreover, diabetes induces lipid peroxidation and increases triglycerides, cholesterol, and LDL-C. Curcumin diminishes lipid peroxidation by normalizing the levels of antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase [31]. The administration of the nano-structure of curcumin spares antioxidant enzymes and improves antioxidant properties, thereby improving the lipid profile.

Induction of diabetes increased the level of liver enzymes, and administration of curcumin decreased their activities. The increased levels of transaminase enzymes, namely ALT and AST, are directly associated with liver cell damage. Augmented levels of these enzymes show hepatic injury in the hepatocellular cytosol and mitochondria [7]. On the other hand, a rise in ALT and AST by curcumin has already been reported in T2DM [8]. Curcumin protects liver hepatocytes against oxidative injuries and decreases the level of antioxidant enzymes.

The current study showed that diabetes decreased body weight, as well as pancreas and heart weight, and administration of curcumin at the level of 80 alleviated the adverse effects of diabetes on pancreas weight. It has been reported that diabetes reduces body weight [32, 33]. Diabetes decreases energy and body weight, increases urinary excretion and catabolic processes [34], and damages the pancreas. Curcumin in any form could not alleviate the adverse effects of diabetes on body weight. Hodaei et al. [35] showed that curcumin supplementation did not improve body weight in patients with T2DM. Consumption of curcumin decreased body weight in patients with metabolic syndrome [36]. It seems that more time was needed to recover the body weight or organ weights disrupted by streptozotocin injection in rats. However, the weight of the pancreas partly improved. It might be needed to optimize the level and time of curcumin or nano-micelle curcumin administration, or diabetes inducers, such as streptozotocin. It is urgently needed to lower the glucose level of diabetic subjects, and protect their endocrine/exocrine secretion balance and curcumin in all cases showed positive effects on adiponectin, ALT, or AST levels. Further research is required to clarify the suitable doses of curcumin and nano-micelle curcumin along with other nutrients in diabetic subjects.

In conclusion, diabetes-induced damage to the pancreas increased the serum glucose, lipid profile, and insulin resistance and decreased adiponectin, liver enzymes, body weight, and pancreas weight. Administration of the nano-micelle form of curcumin improved insulin resistance and the serum concentrations of glucose, insulin, lipid profile, adiponectin, and liver enzymes. It is suggested that curcumin nano-micelle might be effective for diabetic patients with special attention to doses.

Materials and Methods

Experimental animals

All the used procedures were approved by the Ethics Committee of Ferdowsi University of Mashhad, Iran (No: 3.44995). A total number of 60 male Wistar rats with a mean weight of 180-200 g and 60 days of age were purchased from the Pasture Institute (Tehran, Iran). The animals were kept in standard individual cages for 49 days (7 days of adaptation and 42 days of study) under a 12L:12D lighting cycle at 20 °C-25 °C. The animals had free access to standard pellet feed and fresh water. Feed was prepared from Javaneh Khorasan Company (Mashhad, Iran).

Preparation of curcumin and nano-micelle curcumin

Pure turmeric rhizome extract as the powder was purchased from Sami Lab Limited (Bengaluru, Karnataka, India). The powder contained 79.4% curcumin, 17.6% demethoxycurcumin, and 3% bisdemethoxycurcumin. Nano-micelle form of this extract as nano-micelle curcumin was purchased from Exir Nano Sina Co. (Tehran, Iran, IRC: 1228225765). The measured size of nano-micelle curcumin was about 10 nm as described by Hatamipour et al. [19].

Induction of diabetes

At the beginning of the study, diabetes was induced in 50 male Wistar rats by one dose of intraperitoneal streptozotocin (Sigma, St. Louis, MO, USA) (60 mg/kg body weight) in 0.05 M cold citrate buffer (pH 4.5) for the overnight fasted rats. To stabilize streptozotocin in an aqueous media, cold citrate buffer was

used. A group of 10 rats was not treated with streptozotocin and considered healthy control animals. An Accu-chek blood glucose meter (Roche Diagnostics) was used for monitoring fasting blood glucose. The rats with fasting blood glucose higher than 250 mg/dl for five consecutive days after the administration of streptozotocin were considered diabetic rats.

Following diabetes induction, the animals were divided into five groups and received no dietary supplement of curcumin or nano-micelle curcumin (Diab-Con), 40 mg curcumin/kg of pelleted feed (Diab-Cur40), 80 mg curcumin/kg of pelleted feed (Diab-Cur80), 40 mg nano-micelle curcumin/kg of pelleted feed (Diab-Nano40), and 80 mg nano-micelle curcumin/kg of pelleted feed (Diab-Nano80). A group of 10 rats was considered a healthy control and did not receive any streptozotocin or curcumin forms (Healthy-Con). All formulations were fed to rats for 42 days.

Body and organ weight

At the end of the experiment, the animals were weighed and then euthanized by CO_2. Weights of the liver, heart, and pancreas were calculated as the percentage of live body weight.

Blood biochemical parameters

At the end of the study and after 12 h fasting, blood samples were collected from the left ventricle, centrifuged at 2500 RPM for 15 min, stored at -20°C, and investigated for blood biochemical parameters. The serum concentrations of AST, ALT, glucose, insulin, triglycerides, cholesterol, HDL-C, and LDL-C were evaluated by an enzyme-linked immunosorbent assay commercial kit (Pars Azmoon, Tehran, Iran). HOMA-IR index was measured based on the fasting serum glucose concentration (mmol/l) and the fasting blood serum insulin concentration (μU/ml) divided by the constant 5.22×18 as reported by Matthews et al. [20]. The level of adiponectin was assessed by commercial kits (Otsuka Pharmaceutical Co., Tokyo, Japan).

Data analysis

The obtained data were analyzed by the SPSS software version 24 and the significance was designated at $p < 0.05$ for the differences between the six groups for all studied parameters. The differences between all the studied groups were evaluated by a one-way analysis of variance.

Authors' Contributions

H.D. performed the experiment and wrote the main manuscript. H.K. contributed to the experiment as supervisor and corresponding author. M.R.J. and A.J. contributed to the manuscript as advisors.

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Competing Interests

The authors declare that there is no conflict of interest.

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Chronic endometritis-causing bacteria in Arabic mares

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ABSTRACT

Bacterial infections of the uterus are known to be an important cause of infertility in the mare. The objective of this study was to determine the species of bacteria isolated from the uterus of infertile Arabic mares, and investigate how identified bacteria are related to parity. A total of 18 Arabic mares with a history of long-term infertility were evaluated. The age range of mares was 4-23 years. For statistical analysis, logistic regression and Chi-square test were used by Proc Logistic of SAS. In this study, low-volume uterine flush and culture techniques were used. *P. aeruginosa* was the most prevalent bacterium isolated from 26.32% of mares as pure or in conjunction with *E. coli, K. pneumoniae*, or *Citrobacter spp*. Furthermore, 23.68% of bacterial infertility cases were related to *E. coli.* Pure growth of *E. coli* was observed only in one case. However, mixed growth with *P. aeruginosa, S.zooepidemicus,* and *S. aureus* was very prevalent. The present study revealed that the most prevalent bacteria isolated from chronic endometritis in Arabic mares were gram-negative bacteria ($p < 0.05$), while in some cases may be accompanied by gram-positive bacteria. *C. albicans* was isolated in only 8% of mares with chronic endometritis. Moreover, older age and higher parity number of the mares were not related to the presence of intrauterine fluid or the species of bacteria ($p > 0.05$). It can be concluded that the most prevalent bacteria isolated from infertile Arabic mares with chronic endometritis are gram-negative bacteria.

Keywords

mare, uterus, endometritis, bacteria, parity

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Abbreviations

P. aeruginosa: Pseudomonas aeruginosa
E. coli: Escherichia coli
K. pneumoniae: Klebsiella pneumoniae
S. aureus: Staphylococcus aureus
S. zooepidemicus: Streptococcus zooepidemicus
T. equigenitalis: Taylorella equigenitalis
C. albicans: Candida albicans

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Introduction

Despite using sophisticated anti-microbial agents, endometritis is one of the major causes of mare infertility and has been reported to be the third most common medical condition in horses [1, 2]. Bacterial infections of the uterus are known to be an important cause of endometritis and reduced fertility in mares [3]. There is no normal flora in the uterus of cycling mares. If the culture technique is good, any organism isolated from the uterus is a potential cause of infection and infertility [4]. The principal bacterial pathogens involved in endometritis are S. zooepidemicus, *E. coli, P. aeruginosa, K. pneumoniae, S. aureus, and T. equigenitalis*. In addition, the most common fungi responsible for endometritis include *C. albicans* and *Aspergillus* [5].

The uterus is repeatedly exposed to these contaminants at breeding, parturition, and gynecological examinations [5]. However, the uterus has defense mechanisms to clear contamination. These mechanisms consist of anatomic (physical) barriers, cellular phagocytosis, and physical evacuation of uterine contents [6]. There is a rapid migration of neutrophils in the normal uterus which destroys bacteria rapidly within 24 h. Afterwards, inflammatory materials are eliminated mechanically. The absence of defense mechanisms leads to the formation of uterine infections. Moreover, susceptible mares often have fluid remaining in their uterus, and this fluid is evacuated from the uterus with delay. These mares have low fertility because they fail to provide a suitable environment for the growth of embryos [7]. The main point in the successful management of mares with such problems is recognition shortly before or after mating [8, 9]. In order to evaluate the mares with infertility problems, knowing the history of the mares, bacteria isolated from genitalia, age, breed, quality of husbandry, as well as knowledge about the last parturition, and abnormal cycles are important [10, 11].

It should be noted that the frequent occurrence of acute endometritis may cause chronic endometritis with mucociliary dysfunction [12]. In chronic endometritis, biofilm forms in the endometrium that provides an adhesive environment for bacteria. Some bacteria, such as *E. coli*, produce a biofilm that protects itself and other microorganisms from the inflammatory response [13]. Chronic endometritis is related to some factors, such as mare age, cervical problems, and perinea dysfunction [14]. The role of bacteria in chronic endometritis has been proven. The capability of bacteria to cause endometritis results from sticking to the endometrium, inducing inflammation, biofilm production, and resistance to phagocytosis [15]. In broodmares, persistent endometritis is a frequent cause of sub-fertility [12]. Classically, antibiotics act against fast-proliferating bacteria. Therefore, the bacteria outside the biofilm might be damaged, while the bacteria in the center of the biofilm stay alive and lead to chronic endometritis [16].

LeBlanc demonstrated that the most common organisms isolated from chronic endometritis in old mares included *S. zooepidemicus, E. coli, K. pneumoniae, P. aeruginosa, C. albicans,* and *Aspergillus* [13]. This researcher described in 2010 that chronic endometritis is more common in old mares [17]. Some authors have introduced the main cause of acute endometritis as *S. zooepidemicus, E. coli* (haemolytica), *P. aeruginosa,* and *K. pneumoniae* [18, 19].

Clinicians face various problems in impregnating mares with endometritis. Consequently, this study aimed to determine the causes of endometritis resistant to routine treatments. We tried to identify common bacteria involved in chronic endometritis of Arabic mares in Iran using differential and specific culture media. Clinicians can use the results of this study for choosing their treatment strategy. Furthermore, it has been tried to determine the relationship between the type of bacteria isolated from the infectious uterus and the number of parity.

Results

Eighteen Arabic mares with a history of infertility (normal anatomy and physiology but non-pregnant after four matings) were included in the study. The age range of mares was 4-22 years, of which three mares were maiden, seven had 1-5 parities, and eight had 6-13 parities. In estrus, when the dominant follicle was 35-40 mm, the uterine examination by ultrasound showed that the mare had endometrial edema with the accumulation of intrauterine fluid (≥ 2 cm). There was no significant association between the number of parity and intrauterine fluid accumulation. In this study, all the mares had bacterial or yeast growth on the uterus sample in the estrus phase. Figure 1 shows the presence of each endometritis-associated organism in pure or mixed forms. As can be seen, *P. aeruginosa* and *E. coli* were isolated more than others.

P. aeruginosa was the most prevalent isolated bacteria (25% of mares, Figure 1) that could be pure or in combination with *E. coli, K. pneumoniae, or Citrobacter spp.* In one case, *P. aeruginosa* infection was observed along with *S. zooepidemicus* and in another with *S. aureus*. Furthermore, 24% of bacterial infertility was related to *E. coli* (Figure 1). Pure growth of *E. coli* was found only in one case but mixed growth with *P. aeruginosa, S. zooepidemicus*, and *S. aureus* was very prevalent. In one mare, *E. coli* grew with *C. albicans.* The pure growth of *S. zooepidemicus* and *S. aureus* was not seen and all gram-positive bacteria were observed

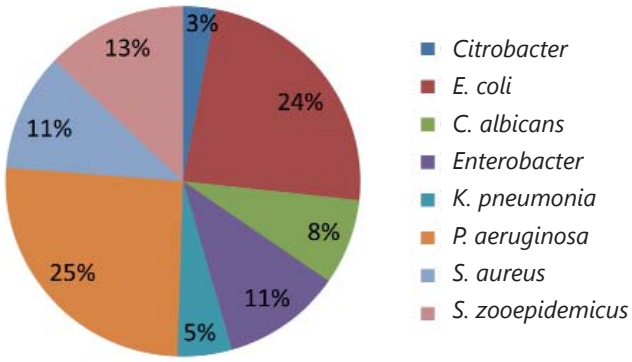

Figure 1.
Percentage of each organism isolated from infertile mares uterus.

Table 1.
Percentage and proportion of bacteria and yeast isolated from the uterus of infertile mares

Type	Gram-negative bacteria	Gram-positive bacteria	*C. albicans*
Pure Growth	7 (39%)	0	0
Mix Growth	11 (61%)	9 (100%)	*3 (100%)*

Table 2.
RResult of Genmod for *C. Albicans* and gram-negative and gram-positive bacteria

Organism	LSM ± SE[1]
C. albicans	$0.91^{a} \pm 0.58$
Gram-negative	$7.88^{b} \pm 0.21$
Gram-positive	$2.73^{a} \pm 0.34$

1) Values indicate least square means ± standard error
2) a, b representative of significant value, $p < 0.05$

mixed with gram-negatives.

From another perspective, gram-negative bacteria were the most prevalent isolated organisms (39% pure growth and 61% with gram-positive bacteria or *Candida*). In this study, no pure growth of *Candida* or gram-positive bacteria was observed. All the growths of *C. albicans* or gram-positive bacteria were mixed with gram-negative bacteria (Table 1). In order to estimate the least squares means, we analyzed the data with PROC GENMOD. The least squares means are presented in Table 2. There was no significant association between the number of parity and the presence of intrauterine fluid or the class of microorganisms in endometritis ($p < 0.05$) (Table 3). The results for analysis of logistic regression on organism classification and parity are presented in Table 4.

In order to examine the independent effect of each variable on endometritis, logistic regression analysis was performed. The analyzed variables included organism classification and parity. Only the variable "organism classification" was found to be related to endometritis ($p < 0.05$). No relationship was found between the number of parity and endometritis ($p > 0.05$). Odds ratios (OR) and their corresponding 95% confidence intervals (CI) were computed for all variables included in the final model (Table 5).

Table 3.
Isolated bacteria and yeast from the uterus of infertile mares in different parity

Organism/parity No.	0	1-5	6-13	Total	Y, N, or P
No growth	0	0	0	0	-
C. albicans	0	2.63	5.26	7.89	Y
Citrobacter spp	0	0	2.63	2.63	N
E. coli	5.26	10.53	7.89	23.68	N
Enterobacter spp	2.63	7.89	0	10.52	N
K. pneumonia	0	0	5.26	5.26	N
P. aeruginosa	0	10.53	15.79	26.32	N
S. aureus	2.63	7.89	0	10.52	p
S. zooepidemicus	5.26	5.26	2.63	13.15	p
Total	15.78	44.73	39.46	100	Yeast + P + N

P: Gram-positive, N: Gram-negative, and Y: Yeast

Discussion

The objective of the present study was to identify the type of bacteria in the uterus of mares with a history of long-term infertility and to assist practitioners' management in endometritis cases. The current study showed that the furthermost rampant bacteria in chronic endometritis of Arabic mares are gram-negative bacteria ($p < 0.05$) sometimes accompanied by gram-positive bacteria. In addition, intrauterine fluid accumulation and bacterial species were not related to the age and parity of mares ($p > 0.05$).

According to the technique described by Katila, a double-guarded method was used for collecting uterine lavage fluid [20]. Blood agar and chocolate agar were used for isolating aerobic and anaerobic bacteria from the collected uterine fluid. According to Brooks et al., blood agar and chocolate agar are complex, non-selective media, which support the growth of different bacteria [21]. Furthermore, MacConkey agar, eosin methylene blue agar, mannitol salt agar, triple sugar iron agar, and C.E.M.O. agar base were used as differential culture media. These differential culture media were selected based on Jawetz medical microbiology textbook [21]. Catalase and oxidase assays were also used to identify gram-positive bacteria. These techniques have been previously described by Murray and others [22].

According to Figure 1, in our study, *P. aeruginosa* was the cause of 25% of chronic endometritis cases in Arabic mares, with *E. coli* in the second place accounting for 24% of chronic endometritis patients. In chronic endometritis cases, the particles of biofilm were observed in the uterine lavage. *P. aeruginosa* is regarded as a venereally transmitted pathogen by some clinicians [23-26]. In one study in Saudi Arabia, *P. aeruginosa* was one of the most common bacteria associated with endometritis in mares, camels, and cows [27]. According to Frontoso et al., 4%-10% of mares and 36% of stallions can harbor P. aeruginosa in their genitalia [28]. It is thought that by completely replacing natural mating with artificial insemination, endometritis caused by *P. aeruginosa* will be reduced.

In 2017, Ryan A. Ferris and colleagues declared that the clinical isolates of *P. aeruginosa* from the equine uterus can produce a biofilm [29]. In other words, *P. aeruginosa* can lead to chronic endometritis that resists treatment. Due to the high prevalence of *P. aeruginosa* in infertile uterine fluid (Figure 1), practitioners should consider these issues when dealing with long-term infertility and uterine fluid in the ultrasonic examination. Because of the venereal transmission of *P. aeruginosa*, we believe that we should not allow mating until complete cure and we strongly recommend that natural mating be replaced by artificial insemination. It is also recommended that all hygiene principles be strictly followed during artificial insemination.

In a study by Frontoso et al., bacteria were isolated from 49% of infertile mares and 18.4% of cases related to *E. coli* [28]. In our study, bacteria were isolated from all mares that remained infertile after four matings. Moreover, in this study, after *P. aeruginosa*, *E. coli* was the second most important bacterium isolated from the uterus of infertile mares. According to the results of the present study and clinical observations, it is thought that most cases of chronic endometritis caused by *E. coli* are related to problems in the perinea.

Gram-negative bacteria, such as *P. aeruginosa* and *E. coli* are highly capable of forming biofilms [21]. A biofilm is the organized life of bacteria within an extracellular matrix [30]. It seems that in our study, biofilm formation by gram-negative bacteria was the main cause of long-term infertility.

Biofilm formation consists of four stages, including initial surface attachment, microcolony formation, formation of biofilm architecture, and biofilm propagation [31]. *S. aureus* biofilm has been observed in cases of contaminated catheters [21], which is likely to cause *S. aureus* to enter the uterus and cause biofilm formation on the endometrium.

Most bacteria isolated from the clinical cases of mare endometritis include *S. zooepidemicus, E. coli, K. pneumoniae,* and *P. aeruginosa* [24]. LeBlanc (2008) showed that K. pneumoniae is one of the main causes of infertility in mares, and has been resistant to most antibiotics [24], but in our clinical study, *K. pneumonia* accounted for 5% of chronic endometritis cases. In the literature, *Enterobacter spp*. was not discussed as the main causative agent of infertility [4, 23, 24], which is inconsistent with the results of our study (10% of all chronic endometritis cases).

In our study, OR estimates showed that the incidence of gram-negative-related endometritis was approximately 20 times higher than the incidence of gram-positive-related endometritis (Table 5). Pure growth of gram-negative bacteria and mix growth were seen in 39% and 61%of infertile mares, respectively. No pure growth of gram-positive bacteria was

Table 4.
Result for the analysis of logistic regression on organism classification and parity

Effect	df	Wald *Chi*-Square	Pr > ChiSq
Organism classification	2	6.4463	0.0398*
Parity	2	3.7371	0.1543ns

* Indicate significant difference in $p < 0.05$ and ns sanded for non-significant effect

Table 5.
Odds ratio estimates for statistical comparison between different investigated factors

Effect	Point estimate	95% Wald confidence limits	
Candida vs gram-positive	0.017	< 0.001	1.134
Gram-negative vs gram-positive	20.453	0.541	773.304
Parity 0 vs Parity 6-13	0.032	< 0.001	1.295
Parity 1-5 vs Parity 6-13	0.712	0.035	14.386

* Parity did not have any significant effect on the type of bacteria isolated

observed in this study (Table 1).

Ferrer et al. [32] reported that the most commonly isolated bacterial species was E. coli (30.7%), and mares with mixed growth most commonly grew a combination of gram-negative and gram-positive bacteria (65.5%). Furthermore, endometritis due to *T. equigenitalis* was reported by many clinicians and researchers [33, 34]. In the current study which was performed on a limited number of mares with chronic endometritis, *T. equigenitalis* was not isolated.

Results of the present study revealed that the most prevalent bacteria isolated from infertile Arabic mares were gram-negative bacteria (68% of the isolated bacteria), and in some cases were accompanied by gram-positive bacteria. Therefore, in mares with a history of long-term infertility, if the clinician does not have access to the microbiology laboratory for an antibiogram, it would be better to use broad-spectrum antibiotics that are more effective against gram-negative bacteria. Furthermore, the findings of this study indicated that the increasing age and parity of mares were not related to the presence of intrauterine fluid or the species of bacteria. In mares with chronic endometritis, mucolytic agents, such as DMSO and N-acetylcysteine, are recommended for biofilm disruption and better effects of antibiotics.

Materials and Methods

Animals

In this study, 18 Arabic mares with a history of infertility were used. The mares were examined by ultrasonography (SIUI CTS-900, equipped with a 5 MHz linear-array transducer Guangdong, China) for ovarian follicle diameter, uterine status, and the existence of uterine fluid. Mares with a history of infertility, uterine fluid accumulation, and ovarian follicles of > 35 mm in diameter (n=18, aged 4-22 years) were enrolled in the study.

Sampling

Primarily, the tails of mares were wrapped and pulled to the side. The vulva was thoroughly washed with detergents. Next, uterine lavage for bacteriological examination was taken. Briefly, an infusion of 60 ml of normal saline into the uterus was taken with a double-guarded catheter. After centrifuging uterine reversal fluid, the pellets were used for microbiological examinations.

Microbiological culture

Samples were cultured directly onto blood agar and chocolate agar and were incubated at 37℃ and 5% CO_2. Bacterial growth was investigated after 24 and 48 h. Afterwards, gram staining was performed on isolated bacterial colonies. In addition to gram staining, catalase and oxidase assays were also used to identify gram-positive bacterial species. The grown bacteria were re-cultured on differential media, namely MacConkey agar, eosin methylene blue agar, mannitol salt agar, triple sugar iron agar, and C.E.M.O. agar base.

Statistical analysis

For statistical analysis, PROC LOGISTIC for logistic analysis was used. The significance level for the Chi-square test was $p < 0.05$. Differences in the presence of different infectious organisms were analyzed using a generalized model with PROC GENMOD. All analyses were carried out using SAS 9.2.

Authors' Contributions

BQP and GM conceived and planned the experiments. BQP and MK carried out the experiments. AR contributed to the interpretation of the results. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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Competing Interests

The authors have no conflict of interest to declare.

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Case Report

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Surgical treatment of right lateral abdominal hernia in a heifer

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ABSTRACT

A lateral abdominal hernia is relatively a less common incidence resulting from abdominal wall defects in cows and heifers than other types of hernias. This is usually an acquired defect, and heifers at the farm and field levels can be affected by accidental trauma or as the sequelae of poorly handled abscesses or wounds in the abdominal region involving the rupture and tear of the regional muscular intersections. An affected heifer was clinically examined, and an oval-shaped swelling on the right lateral side of the abdomen and cranial to the stifle skinfold was detected with a palpable sac having the characteristics of a prominent ring and reducible herniated mass covered by subcutis and intact skin. Being diagnosed as a right lateral abdominal hernia, the case was further surgically treated by herniorrhaphy to reconstruct the abdominal wall defects. Postoperatively, the animal was provided with intensive care and supportive medications. There are several reports on ventrolateral and ventral abdominal hernias in small ruminants; however, case studies focusing on the clinical diagnosis-based treatment of right lateral abdominal hernia in large ruminants have rarely been reported.

Keywords

abdominal muscle tear, heifer, herniorrhaphy, ovoid swelling, reducible hernia

Abbreviations

BW: Body Weight
NaCl: Sodium chloride
Ltd: Limited

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Introduction

A hernia is the protrusion or displacement of an organ or part of tissue outside the body cavity through an unusual opening in the cavity wall which can be detected as a bulge of skin externally [1-3]. This opening is often due to the rupture of the abdominal muscles in accidents, or it seems to be a natural orifice similar to the femoral and inguinal canals. Various types of hernias are found in both small and large animals which can be categorized according to the anatomical locations, such as abdominal (lateral/ventral), incisional, inguinal, umbilical, diaphragmatic, femoral, scrotal, and perineal hernias [4-8]. In addition, hernias can also be classified into reducible and irreducible forms.

In the case of a lateral abdominal hernia, the abdominal contents protrude through an unusual orifice of the abdominal wall, and the hernia is lateral to the stifle skinfold [7]. A hernia is the most common abdominal disease in newborn calves and it is also found in heifers and other ruminants. However, the livestock farmers of rural communities often ignore this condition unless severe complications occur [9]. Several causes are associated with the development of an abdominal hernia. A lateral abdominal hernia is mainly caused by trauma due to horn thrust, animal kicks, blunt objects, jumping, falling, external force, automobile accidents, abscess in the abdominal wall, and weakening of the musculatures [2]. Moreover, congenital defects may also induce herniation in some cases [7]. Lateral abdominal hernias are usually acquired in origin just like the ventral abdominal hernias in ruminants and other species [9].

In animals, hernia reduces performance and production [7, 10] along with lowering the market value. Clinically the affected animal may present severe discomfort, pain, inappetence, and loss of body weight [2]. Diagnosis is often based on clinical inspection and palpation of the hernial contents and ring. However, radiographic imaging is used to detect the abdominal wall continuity in case of irreducible hernias [11]. Differential diagnoses may include localized cysts, abscesses, tumors, neoplastic growth, and various inflammatory swellings [2].

Treatment of hernias should be as soon as possible, otherwise, severe complications, such as incarceration and strangulation of the bowel, may arise, and the prognosis will be very poor and life-threatening. Exploratory laparotomy followed by herniorrhaphy is the most preferred surgical approach to treat this defect. In addition, the use of various types of synthetic mesh for hernioplasty is necessary when the hernial ring becomes enlarged in diameter [12]. The present study highlights the surgical correction of a right lateral abdominal hernia in a heifer through traditional herniorrhaphy.

Case Presentation

A crossbred heifer of 182 kg BW aged above 2 years was referred to the Veterinary Teaching Hospital of Bangladesh Agricultural University with the complaint of an almost round and projected type swelling in the right side of the abdomen (Figure 1A). Clinical history included previous treatment by quack for a complicated abscess by incisional drainage and, as a consequence, this lesion had emerged after a few days.

Clinical examination revealed an ovoid-shaped protruding mass covered by the skin on manual palpation with voluminous and reducible swelling along with the feeling of bulging out abdominal contents due to muscular rupture at the right side of the abdominal region having 13.5 cm length and 9 cm width (Figure 1B). It was just in a ventral-oblique position to the last four ribs and parallel and cranio-lateral to the stifle skinfold. In addition, the animal showed anorexia and apathy without any pain and systemic illness. There was a gradual increase in the size of the swelling reported by the animal owner. Based on these clinical details, the case was eventually diagnosed as a right lateral abdominal hernia.

No further diagnostic imaging was performed, and reconstruction of the abdominal wall with herniorrhaphy was decided to handle the case. The heifer was kept fasting for 12 h, and 5% dextrose in normal saline (0.9% NaCl) was administered intravenously to correct dehydration before the operation. Next, the animal was restrained in left lateral recumbency with upper exposure of the hernia being premedicated intramuscularly with atropine sulfate (Atrovet®, Techno Drugs Ltd., Narsingdi, Bangladesh) at the dose of 0.04 mg/kg BW, followed by intramuscular sedation with xylazine hydrochloride (Xylaxin®, Indian Immunologicals Ltd., Hyderabad, India) at the dose of 0.1 mg/kg BW. Presurgical aseptic procedures were completed to disinfect the skin over the defected area with 10% povidone-iodine (Viodin® 10% Solution, Square Pharmaceuticals Ltd., Dhaka, Bangladesh) after gently shaving the hairs to prepare the surgical site. Linear infiltration of 2% lidocaine hydrochloride (Jasocaine®, Jayson Pharmaceuticals Ltd., Dhaka, Bangladesh) was performed at the surgical site to achieve local anesthesia.

For herniorrhaphy, a linear and slight oblique incision was made on the skin over the area of local infiltration. Afterwards, the skin was everted, and the underlying tissues and muscles were bluntly dissected to expose the hernial sac and ring (Figure 2A). The hemorrhage was checked carefully, and the sac was incised cautiously leaving the inner content uninjured, and

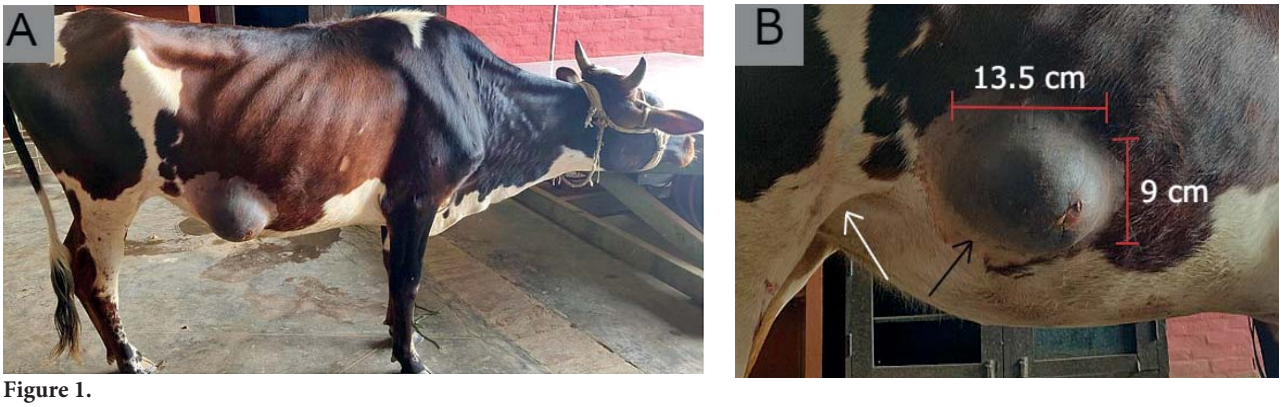

Figure 1.
A) Right lateral view of the affected heifer. B) Ovoid swelling (black arrow) on right lateral abdomen cranially to stifle skinfold (white arrow).

thereafter the contents were reduced back to the abdominal cavity (Figure 2B) ensuring no adhesion and complication. Next, the excess portion of the sac was discarded just near the hernial ring (Figure 2C), and the ring was closed properly with a simple interrupted suture using Polyglactin 910 of size-1 (Vicryl™, Ethicon, J & J Medical Devices Companies, United States) having the accurate judgment of the persistent pressure of bowel (Figure 2D).

First, two simple interrupted knots were applied at the two furthest edges of the elliptical hernial ring to provide support during the placement of the rest of the sutures. A series of simple interrupted sutures were placed through the edges of the ring. The ends of the sutures were pulled, tightened, and secured by starting from the center towards each of the commissures. Another layer of simple interrupted suture was placed over the previous one covering the associated muscles to make them more secure and stable (Figure 2E). Then, the skin was rationally closed with a horizontal mattress suture modified with intermittent simple interrupted knots using Nylon threads (Figure 2F) after discarding excess loose portions. Finally, a cotton seal soaked with Viodin® 10% solution was topically applied over the suture lines (Figure 2G) to prevent contamination, and the animal was on its feet within an hour after surgery (Figure 2H). However, daily feeding with a one-half regular diet and restricting the excess movement of the animal for 3 weeks were recommended. In addition, the animal was monitored carefully with the regular administration of supportive medications, including appropriate courses of ceftriaxone at the dose of 15 mg/kg BW (Trizon Vet, ACME Laboratories Ltd., Dhaka, Bangladesh) twice daily for twelve days, ketoprofen at 3.3 mg/kg BW (Ketovet, Techno Drugs Ltd., Narsingdi, Bangladesh) once daily for four days, and pheniramine maleate at 1 mg/kg BW (Antihista-Vet®, Square Pharmaceuticals Ltd., Dhaka, Bangladesh) once daily for seven days along with strict hygienic

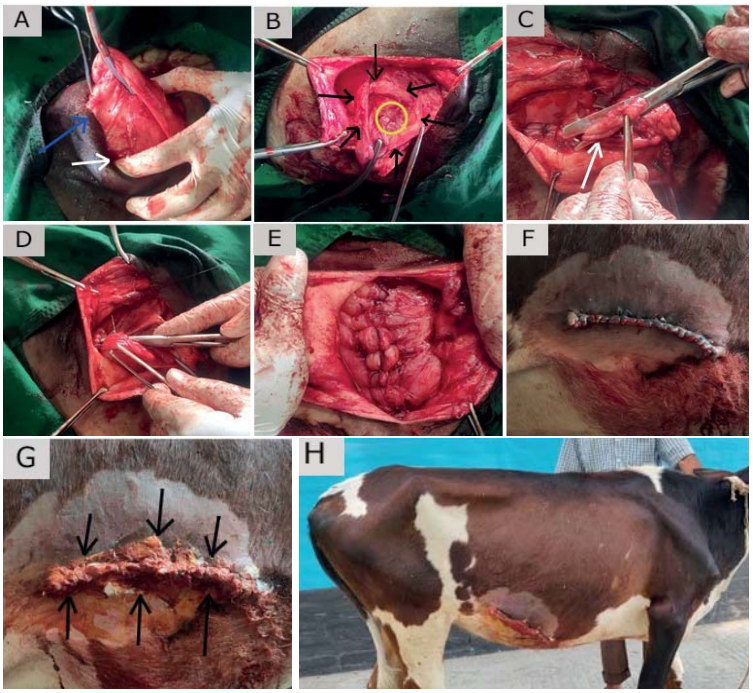

Figure 2.
A) Exposed hernial sac (blue arrow) and ring (white arrowed finger point). B) Incised sac (black arrows) indicating reduced bowel (yellow circle) back to abdomen. C) Discarded sac residuals (white arrow). D) Closure of hernial ring. E) Muscle closure. F) Skin closure. G) Medicated cotton seal (black arrows) applied on suture lines. H) Heifer after 45 minutes of surgery.

measures for better healing and recovery. Fly repellent was routinely used in the animal shed to prevent myiasis. Two days after surgery, 5% povidone-iodine (Viodin® 5% Ointment, Square Pharmaceuticals Ltd., Dhaka, Bangladesh) was topically applied twice daily for the next seven days. On the 14th postoperative day, the external sutures were removed.

Results and Discussion

In the present study, a right lateral abdominal hernia was clinically found in a heifer at the field level, which was similar to the reports of others [5, 13]. Right-sided ventral and left-sided ventrolateral hernias in abdominal regions have previously been found in various species [4, 14-16]. Moreover, these types of cases are also found in cows, buffaloes, sheep, and goats [2, 4, 11]. Ovine species have the highest percentage of hernias, followed by caprine and bovine species [14]. The mentioned heifer presented an ovoid-shaped swelling covered by skin, which contained the adjacent intestinal segments internally, and was located at the right latero-ventral side of the abdomen resembling several previous cases [5, 6, 8, 9].

According to the owner, the hernia had developed due to the mismanagement of an abscess by a local quack. This might be attributed to the tearing and rupture of the external and internal obliquus abdominis, rectus, and transversus abdominis muscles during a faulty process of pus evacuation leading to a passage for protruding the voluminous bowel segments out of the cavity and underneath the subcutaneous tissues. The other causes reported for abdominal hernias are different types of external and accidental injuries, increased intra-abdominal pressure during pregnancy, loss of abdominal wall strength with age, and weakness of the abdominal muscles and tendinous intersections due to malnutrition [15, 16].

The described case was corrected by surgical interventions using herniorrhaphy, as also observed in other research [7, 17]; however, hernioplasty is conventionally used to manage extensive abdominal hernias [6, 10, 12]. Furthermore, there are several conservative treatments including the application of bandages, clamps, or ligatures, which may be helpful for the ventral and reducible abdominal hernias with smaller hernial rings [18].

This case presented a large hernial ring which was closed by suturing. However, in general, such type of large opening cannot be closed by suturing and requires mesh grafting. This was possible due to the availability of enough surrounding muscle flaps fairly stretched out to enclose the opening adequately during the operation. The closure of the hernial ring involved firstly, the insertion and placement of sutures through the edges of the ring, followed by adequate tightening to deal with the steady pressure of the bowel. Another reason for this was to avoid any adhesion or entrapping of the bowel segment(s) during the whole suturing process to close the ring as it provided the ease to observe the suture lines and bowel segment(s) during pulling the suture ends and tying the knots.

The use of atropine sulfate and xylazine hydrochloride for premedication and sedation, and 2% lidocaine hydrochloride for local anesthesia in this study are in agreement with other research findings [3, 19, 20]. In this case, Polyglactin 910 of size-1, a synthetic absorbable suture material, was used as internal sutures for muscle closure in a simple interrupted pattern, and Nylon thread was used for skin closure in a horizontal mattress pattern, which were similar to several other reports [3, 11, 21]. In addition, various types of suture materials, such as polyglycolic acid or polydioxanone with simple interrupted, simple continuous, interrupted cruciate, overlapping mattress, and tension-relieving suture patterns have been used for closing hernial ring as well as abdominal wall defects [2, 21, 22].

This case experienced no further complications, and the animal gradually recovered one month after surgery. However, in some reports, postoperative complications, such as wound infections, abscesses, prosthetic infections, seroma, hematoma, and hernial recurrences have been found [23, 24]. The absence of such complications, in this case, might result from appropriate supportive medications and routine postoperative care and management. In conclusion, the right lateral abdominal hernia in the heifer can be successfully treated by surgical repairing with herniorrhaphy depending on the accessibility of muscle closure by suturing.

Authors' Contributions

MRM performed surgery, case follow-up and manuscript writing. RIM and ST performed clinical examinations, review literature and manuscript draft.

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Competing Interests

The authors declare that they have no competing interests.

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Abstract in Persian

تشخیص مولکولی و آنالیز فیلوژنتیکی بویکولا کاپره در غرب و شمال غرب ایران بر اساس نشانگر سیتوکروم اکسیداز ۱

خدیجه صیدی گزافی[۱]، موسی توسلی[۱]*، کریم مردانی[۲]

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چکیده

شپش ها انگل خارجی دائمی و اجباری پرندگان و پستاندارانند. شپش بویکولا کاپره، شپش جونده بز سبب ایجاد ازدیاد حساسیت، تحریک پذیری، درماتیت، کم خونی، کاهش وزن وکاهش تولید در بز می شود.این مطالعه به منظور مقایسه مولکولی بویکولا کاپره بر اساس ژنوم میتوکندریایی در غرب وشمال غرب ایران انجام شد. در مجموع ۱۰۱۷ نمونه شپش جونده بز از پنج استان واقع در غرب و شمال غرب ایران جمع آوری شد و با استفاده از کلیدهای تشخیص تعیین هویت شدند. پس از استخراج DNA و انجام واکنش PCR نمونه ها برای تعیین توالی ارسال شدند. نتایج بررسی مورفولوژیک با بررسی های مولکولی مطابقت داشت. همردیف سازی توالی نوکلئوتیدی نمونه های جداسازی شده از شهرهای مختلف بر اساس ژن سیتوکروم اکسیداز ۱ تشابه درون گونه ای ۱۰۰ درصد را نشان داد. در ترسیم درخت فیلوژنتیک توالی بویکولا کاپره جداسازی شده در این مطالعه در کنار نمونه-های کانادا و چین در یک شاخه قرار گرفتند که بر مبنای ماتریس شباهت بیش از ۹۰ درصد قرابت فیلوژنتیک داشتند. نتایج تجزیه و تحلیل ژن سیتوکروم اکسیداز 1 در این مطالعه نشان داد که این قطعه برای نشان دادن شباهت درون گونه ایی،تمایز در سطح گونه و جنس بویکولا کاپره مفیداست.

واژگان کلیدی

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Abstract in Persian

تغییرات فصلی در سطح پروژسترون سرم در مادیان های کاسپین

رضا حق دوست[1]، محمد روستائی علی مهر[1]،احمد قربانی[2]

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چکیده

این بررسی به منظور تعیین فعالیت تولیدمثل فصلی در اسب کاسپین با استفاده از ۱۰ مادیان در طول ۱ سال انجام شد. مادیان‌ها به سه گروه سنی۳ تا ۵ سال (جوان)، ۶ تا ۷ سال (میانسال) و ۱۴ تا ۱۹ سال (مسن) تقسیم شدند. نمونه‌های خونی (n = ۵۳۰) به صورت هفتگی جمع آوری شدند. فعالیت تخمدان از طریق غلظت پروژسترون ارزیابی شد. غلظت پروژسترون سرم مادیان‌ها که به طور مداوم بیشتر و کمتر از ۱ ng/ml بودند به ترتیب به عنوان شروع فعالیت تخمدان (آغاز فصل تولیدمثل) و توقف فعالیت تخمدان (پایان فصل تولیدمثل) در نظر گرفته شد. نتایج نشان داد که بین زمان و سن بر غلظت پروژسترون سرم، فعالیت تخمدان، وزن بدن و امتیاز وضعیت بدنی رابطه متقابل وجود دارد (p <۰/۰۵). در ماه مارس، غلظت پروژسترون، امتیاز وضعیت بدنی و فعالیت تخمدان در مادیان های جوان بیشتر بود (به ترتیب ۴۶/۶۶ ٪ ± ۸/۶۲، ۴/۶۶ ± ۰/۱۹، ۴/۹۰ ± ۱/۱۵ ng/ml) غلظت پروژسترون، امتیاز وضعیت بدنی و فعالیت تخمدان مادیان های مسن در ماه اکتبر (به ترتیب ۰۰/۱۰۰ ٪ ± ۷/۴۷، ۰۰/۶۰ ± ۰/۱۶، ۹/۰۸ ± ۱/۱۲ ng/ml)، نوامبر (به ترتیب ۵/۸۵ ٪ ± ۷/۴۷، ۵/۵۰ ± ۰/۱۹، ۹/۶۵ ± ۱/۱۲ ng/ml) و دسامبر (به ترتیب ۵/۴ ± ۰/۱۹، ۰۰/۴۰ ± ۱/۱۲ng/ml، ۷/۴۷ ± ۲۵/۰۰٪) در مقایسه با مادیان های جوان و میانسال بیشتر بود (p <۰/۰۵).. بیشترین فعالیت تخمدان مادیان های جوان، میانسال و مسن به ترتیب از آوریل تا آگوست (چهار ماه)، مه تا اکتبر (پنج ماه) و ژوئن تا دسامبر (شش ماه) بود (p <۰/۰۵). درنهایت، فصل تولیدمثل در مادیان های جوان کاسپین در مقایسه با مادیان های مسن دو ماه کوتاه تر و شروع فصل تولیدمثل زودتر بود.

واژگان کلیدی

اسب کاسپین، فصل، تولید مثل، پروژسترون

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Abstract in Persian

تاثیر کورکومین و نانومیسل فرموله شده آن بر وزن بدن، مقاومت به انسولین، آدیپونکتین، و پارامترهای بیوشیمیایی خون موشهای دیابتی شده با استرپتوزوتوسین

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چکیده

اثر کورکومین و نوع نانومیسل آن بر وزن بدن، مقاومت به انسولین، آدیپونکتین و پارامترهای بیوشیمیایی خون موش های دیابتی شده با استرپتوزوتوسین بررسی شد. پنجاه موش ویستار دیابتی شده به پنج گروه ده تایی با تیمارهای ۱) بدون مکمل در جیره، ۳-۲) ۴۰ و ۸۰ میلی گرم کورکومین در کیلوگرم جیره، ۵-۴) ۴۰ و ۸۰ میلی گرم نانومیسل کورکومین در کیلوگرم جیره تقسیم شدند. یک گروه ده تایی موش ویستار، دیابتی نشده و به عنوان شاهد سالم در نظر گرفته شد.غلظت سرمی AST ، ALT، گلوکز، انسولین، انسولین مقاوم، تری گلیسرید، LDL ، HDL کلسترول و آدیپونکتین اندازه گیری شد. وزن بدن، قلب و کبد، و پانکراس نیزبررسی شد.سرم موشهای دیابتی شده مقدار بیشتری از ALT و AST، گلوکز، تری گلیسرید LDL و انسولین مقاوم را نشان داد. غلظت سرمی ، انسولین، آدیپونکتین، کلسترول HDL، وزن بدن و وزن قلب و پانکراس افزایش یافت ($p < ۰.۰۵$) .کورکومین نانومیسل شده تاثیرات منفی دیابت را در موشها برای گلوکز، پروفیل لیپیدی و آنزیمهای کبدی بهبود داد ($p < ۰.۰۵$) .نتیجه اینکه فرم نانومیسل کورکومین در مقایسه با کورکومین می تواند اثرات منفی دیابت را در موشها بهبود دهد. توصیه اینکه در دزهای خاصی می توان از نانومیسل کورکومین برای درمان دیابت استفاده کرد.

واژگان کلیدی

کورکومین ، نانوکورکومین ، دیابت ، مقاومت به انسولین ، آنزیم های کبدی

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Abstract in Persian

بررسی باکتری های شایع مسبب اندومتریت مزمن در مادیان های عرب

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چکیده

عفونت های باکتریایی رحم از مهمترین علل ناباروری در مادیان محسوب می شوند. هدف از مطالعه حاضر مشخص کردن گونه های باکتریایی مسبب عفونت های رحمی در مادیان های نابارور و بررسی ارتباط گونه باکتری حاضر در رحم عفونی با تعداد شکم زایش در مادیان ها بود. در این مطالعه، تعداد ۱۸ رأس مادیان با تاریخچه ناباروری طولانی مدت ارزیابی شدند. سن مادیان ها ۴ الی ۲۳ سال بود. از مدل رگرسیونی لجستیک و کای اسکوئر برای آنالیز داده ها استفاده شد. شایعترین باکتری جدا شده از عفونت های رحمی مادیان های نابارور پزودوموناس آئروژینوزا بود (۲۶/۳۲ درصد مادیان های مورد مطالعه) که به صورت خالص و یا همراه با ای. کولای، کلبسیلا پنومونیه یا سویه های سیتروباکتر جداسازی شد. همچنین ۲۳/۶۸ درصد موارد ناباروری مربوط به ای. کولای بود. ای. کولای خالص فقط در یک مورد مشاهده شد ولی عفونت همزمان ای. کولای با پزودوموناس آئروژینوزا، استرپتوکوکوس زئواپیدمیکوس و استرپتوکوکوس آرئوس شایع بود. همچنین در مطالعه حاضر مشخص شد که حضور مایع رحمی و گونه باکتریایی با افزایش سن و تعداد شکم زایش مرتبط نمی باشد ($p>۰/۰۵$).

واژگان کلیدی

مادیان، رحم، اندومتریت، باکتری، شکم زایش

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Author Index

Author index

Guide for authors

SCOPE

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Results indicate the results of an original research in a clear and logical sequence. Do not repeat data that are already covered in tables and illustrations. In manuscripts describing more than one animal, all animals should be assigned a case number.

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Discussion should include the answer to the question proposed in the introduction and empha-

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Materials and methods should be described in sufficient details to allow other researchers to reproduce the results. Specify any statistical computer programs used .The methods of data collection and use of statistical analysis will be checked by the referees and if necessary, a statistician. Drugs and therapeutic agents, reagents, softwares and equipments should be given in the format: name (trade name, manufacturer name, city, country), e.g. Statview 5 (SAS Institute, Inc., Cary, NC, USA).

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Gene names: The standard gene names, as provided by HGNC (HUGO Gene Nomenclature Committee) should be used. Gene names must be italicized. If the case of mammalian species and if gene names refer to rodent species, they must be upper case; if they refer to non-rodent species they must be written in capitals. If they refer to other species, they must written lower case. Protein names are written in capitals and are not italicized. As an example:

Mouse beta actin gene: *Actb*
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Quantitative PCR: If the quantitative PCR method has been used, the related section in Materials and Methods must be written following the reference:

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009 Apr;55(4):611-22.

The following information must be provided in the section:

Protocol for DNA/RNA extraction, including quantification and determination of purity;

Reverse transcription (if used): amount of RNA, concentration of all reagents: primers concentration (either random primers or oligonucleotides), reverse transcriptase and master mix components;

qPCR: sequence of forward and reverse primers, probes, amplicon size, accession number of Genebank; thermocycler parameters (i.e. denaturation, annealing and extension steps, number of cycles, melting curves); validation of PCR products; non-template controls for reverse transcription and qPCR should be included in all reactions; and

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An unhealthy diet, obesity and physical inactivity play a role in the onset of type 2 diabetes, but it has been shown that increased physical activity substantially reduces the risk [1], and participation in regular physical activity is one of the major recommendation of the evidence based guidelines for the primary prevention of diseases [2]. According to the 2004-05 National Health Survey, more than half a million Australians (3.5% of the population) have diabetes mellitus which had been medically diagnosed and most of these people have the Type 2 condition [3]. Gestational diabetes is also on the increase, rising steadily between 2000-01 and 2005-06 [4]. Approximately two thirds of those with diabetes have been prescribed medication [3], but it is of concern that a recent review of the literature found that many people do not take their medication as prescribed [5]. Many patients also self monitor the disease by measuring their blood glucose levels with a glucose meter but Song and Lipman [6] have concerns about how well this is managed.

References for the above example:

1. Hull J, Forton J, Thompson A. Paediatric respiratory medicine. Oxford: Oxford University Press; 2015.

2. Eckerman AK, Dowd T, Chong E, Nixon L, Gray R, Johnson S. Binan Goonj: bridging cultures in Aboriginal health. 3rd ed. Chatswood, NSW: Elsevier Australia; 2010.

3. Johnson C, Anderson SR, Dallimore J, Winser S, Warrell D, Imray C, et al. Oxford handbook of expedition and wilderness medicine. Oxford: Oxford University Press; 2015.

4. McLatchie GR, Borley NR, Chikwe J, editors. Oxford handbook of clinical surgery. Oxford: Oxford University Press; 2013.

5. Petitti DB, Crooks VC, Buckwalter JG, Chiu V. Blood pressure levels before dementia. Arch Neurol. 2005; 62(1):112-6.

6. Liaw S, Hasan I, Wade, V, Canalese R, Kelaher M, Lau P, et al. Improving cultural respect to improve Aboriginal health in general practice: a multi-perspective pragmatic study. Aust Fam Physician. 2015; 44(6):387-92.

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Weissgerber TL, Milic NM, Winham SJ, Garovic VD. Beyond bar and line graphs: time for a new data presentation paradigm. PLoS Biol. 2015; 13(4):e1002128.

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PUBLICATION ETHICS

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3. Final approval of the version to be published; AND
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Peer Review Process

PEER REVIEW PROCESS

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Iranian Journal of Veterinary Science and Technology

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