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ON THE COVER

Strongylid egg in rabbit feces (note the thin-shelled wall and the presence of the blastomeres (photo taken by Dr. M. Noormonavvar).; see page 4.

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TABLE OF CONTENTS

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RESEARCH ARTICLE

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A survey on the gastrointestinal parasites of exotic companion species in Tehran, Iran

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ABSTRACT

Exotic pet owners, ranging from small mammals to reptiles, comprise a considerable portion of veterinary clients. Parasitic infections are a threat both for the animal health and the health of the owner. This study aimed to investigate the gastrointestinal parasite species commonly encountered in exotic animal veterinary practice. Exotic pets' fecal samples were examined for fecal parasites macroscopically and microscopically by saline fecal smears, fecal floatation and specific staining. Chi-squared test to investigate the association between the presence and type of the parasites with host species and clinical symptoms ($p < 0.05$). Three hundred fecal samples, including 262 from small mammals, 37 from reptiles and 1 from primate were investigated for gastrointestinal endoparasites. The exotic pet species consisted of Lagomorpha (189/300; 63%), Rodentia (68/300; 22.66%), Reptilia (37/189; 12.33%), Eulipotyphla (4/300; 1.3%), a sugar glider and a marmoset. Thirty-nine samples were found to be infected with at least one gastrointestinal parasite (13%). Parasites observed in the feces of exotic pets included oocysts, strongyle-shaped eggs, oxyurid eggs (*Passalurus ambiguous*) and cestode eggs. A sample from a guinea pig was diagnosed to be infected with *Cryptosporidium sp*. There was no significant association between clinical symptoms and host species with parasite infection ($p > 0.05$). Considering the continuous species alteration, the unidentified sources of the pets in the market, and the potential of zoonotic infections periodical surveys on the common pet species and their parasitic infection are inevitable. Subclinical intestinal parasites in pet animals may alter the well-being of the companion animal if adjoined with poor management. Usually there is no need for anti-parasitic therapy in an animal without clinical signs, but regular diagnostic tests for parasites are advisable for effective veterinary practice.

Keywords

Companion animals, Helminths, Eimeria, Cryptosporidium, Exotic pet

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Abbreviations

sp.: species (singular)
spp.: species (plural)
P. ambiguous: Passalurus ambiguous
MZN: Modified Ziehl-Neelsen

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Introduction

Despite the slight differences in the comprehending exotic pet species worldwide, non-domestic species other than dogs and cats can generally be called exotic species [1]. Despite the proven benefits of pets for human well-being, it is impossible to ignore potential complications such as bites, allergies, and zoonotic diseases. [2]. In addition to the reported viruses, bacteria, and fungi of exotic animals, ectoparasites, and endoparasites, such as helminths and protozoa, can be hazardous to the animal's and the owner's health. [3, 4]. Parasites can be pathogenic, though some may be present without clinical sings. The close contact of companion animals with the owners, besides the inadequate health awareness and the health status of the owners, will alter the companion to the foe [5, 6]. A considerable percentage of referrals to veterinarians are owners of exotic species, which highlights the necessity of continuous education and surveys on the disorders and pathogens of these species. In addition, the diversity and ratio of common pet species and their pathogens vary with time. Exotic pet parasitic surveys revealed the presence of diverse helminth and protozoan parasites, though the results differ with the studied hosts and diagnostic methods. The present study attempts to identify the diversity and frequency of exotic pet intestinal parasites in Tehran.

Result

In the current study, 300 samples derived from two classes (Mammalia and Reptilia) and seven orders were studied for the presence of gastrointestinal parasites. The most abundant exotic pets belonged to mammalians (263/300: 87.7%), in which rabbits (*Oryctolagus*) (189/300; 63%), guinea pigs (*Cavia*) (33/300: 11%) and hamsters (*Mesocricetus*) (19/300: 6.3%) were the most abundant species, respectively. The investigated host species are summarized in Table 1.

In the current study, 13% (39/300) of the examined samples harbored parasites. Except for the 2 tortoise samples, the remaining infested samples were from mammalian species. Rabbits (27/189: 14.3%) were infected with oxyurid eggs (*Passalurus ambiguus)*, eimerian oocysts, and strongylid nematode eggs. One of the rabbits was concurrently infected with oocyst and strongylid eggs. Hamsters (6/19: 31.6%) were found to be infected with nematode, cestode eggs, and eimerian oocysts. One of them was simultaneously infected with cestode and nematode eggs. In the samples from the guinea pigs, apicomplexan protozoa, including Eimerian oocysts and oocysts of *Cryptosporidium sp.*, were detected (3/33: 9%). One of the samples from the investigated squirrels (1/16: 6.2%) contained *Eimeria* sp. oocysts. Other mammalian species, including a sugar glider, a marmoset, and four hedgehogs were not infected. The investigated Squamata were free of fecal parasites, though two of the 16 investigated Testudines (12.5%) harbored Eimerian oocysts. The most frequent parasite was Eimerian oocysts (70%), followed by strongylid eggs (17%), oxyurid eggs (7.3%) and rarely cestodes and *Cryptoporidium sp*. (2.4%) (Figure 1). The results are summarized in Table 1.

A hamster with simultaneous nematode and cestode infection and a rabbit with *oocysts* and nematodes were the hosts with multiparasitism.

It should be noted that most of the referral animals had routine checkups and/or veterinary health care and wellness information. 73% of the rabbits, 89% of the hamsters, 76% of the guinea pigs, 69% of the squirrels, 75% of the testudines, 71% of the lizards, and all of the investigated snakes, hedgehogs, the marmoset, and the sugar glider did not have any clinical symptoms and souk veterinary advice for routine and responsible pet care. Clinical symptoms such as anorexia and lethargy were observed in four cases of rabbits infected with oocysts. There was no significant association between clinical symptoms and parasite infection ($p > 0.05$).

Discussion

The present study included an investigation of gastrointestinal parasites in 300 exotic pets. The investigated hosts included 262 small mammals, 37 reptiles, and one primate. Thirteen percent (39/300) of the examined samples harbored parasites. Nematode eggs including oxyurids and strongylids, cestode eggs, *Eimeria* sp., and *Cryptosporidium* sp., were the detected parasites. Parasitologists worldwide have reported comparable parasitic species in their studies., though variations in the frequencies are evident due to different host species and diagnostic methodologies.

In the current study rabbits harbored oxyurid (*Passalurus ambiguus*), strongyle nematode eggs, and Eimerian oocysts. *Eimeria* oocysts, cestode egg, *Trichusis* sp., *Trichostrongylus*, and *P. ambiguous* had been reported in pet rabbits. A retrospective study on pet rabbits in Nigeria reported mange as the most frequent parasitosis. Helminthic infections and coccidiosis were in the next rows, respectively. The retrospective type of study in which the hospital database was used for data extraction and analysis may literally define the difference. In most studies on rabbits, oocyst infection has been the most commontype of gastrointestinal parasitic infection [8-12], however, the infection is mostly subclinical, causing little to no health

Table 1.
Frequency of host species and identified parasites in the fecal samples of pet exotic species.

Class	Order	Host	Number (%)	No. Infected (%)	Detected parasites
Mammalia	Lagomorpha	Rabbit (*Oryctolagus cuniculus*)	189 (63.0)	27 (14.3)	Oocysts, Strongylid eggs
	Rodentia	Guinea pig (*Cavia* sp.)	33 (11.0)	3 (9)	Oocysts, *Cryptosporidium* sp.
		Hamster (*Mesocricetus auratus*)	19 (6.3)	6 (31.6)	Oocysts, Strongylid eggs, Cestode eggs
		Squirrel (*Sciurus sp.*)	16 (5.3)	1 (6.2)	Oocysts
	Eulipotyphla	Hedgehog (*Erinaceus concolor*)	4 (1.3)	0	-
	Primates	Marmoset (*Callithrix* sp.)	1 (0.3)	0	-
	Diprotodontia	Sugar glider (*Petaurus breviceps*)	1 (0.3)	0	-
Total			263 (87.7)	37 (14.1)	-
Reptilia	Squamata	Snakes	13 (4.3)	0	-
		Iguana (*Iguana iguana*)	7 (2.3)	0	-
		Monitor lizard (*Varanus griseus*)	1 (0/3)	0	-
	Testudines	Tortoises (*Testudo* sp.)	16 (5.3)	2 (12.5)	Oocysts
Total			37 (12.3)	2 (5.4)	-
	Total		300 (100)		

complications. Hamsters are reported to be infected with nematode, cestode eggs, and Eimerian oocysts. In several studies, infection with the zoonotic cestode *Hymenolepis nana*, has been reported in pet rodents, and hamsters were infected more heavily than other pet rodent species [13-16]. In the present study guinea pigs were infected with oocysts and *Cryptosporidium* sp. In studies conducted on pet and household guinea pigs, protozoa, including oocysts, *Trichomonas*, and *Giardia*, helminthic infections, including *Paraspidodera* uncinate, and *Nippostrongylus*-like eggs have been reported [2, 17]. Guinea pig-adapted *Cryptosporidium* species have been assumed as a potential zoonotic agent [18, 19]. Investigated squirrels in the present study were mostly uninfected, and only an oocyst infection was identified. Various parasites, including *Dicrocoelium dendriticum, Syphacia* spp., *Nippostrongylus,* the *zoonotic Capillaria* sp., and different species of *Eimeia* have been reported from pet squirrels [20, 21]. Other exotic mammals investigated in the current study, such as sugar glider and hedgehog, were not infected (Table 1). However, there are reports of parasitosis in accidentally-killed hedgehogs harboring various species of parasites, including *Physaloptera* as a vector of the zoonotic *Leptospira* spp. and *Cryptosporidium* sp. [22-24]. Sugar gliders were reported to harbor parasites acquired from the wild or transmitted in captivity from various sources, including food or the immediate environment [25, 26]. Among all the examined reptile feces, only two turtles were found infected with nematodes, and no protozoan infection was observed (Table 1). *Entamoeba* sp., *Cryptosporidium* sp., *Isospora* sp., and *Eimeria* sp. and various helminths have been reported in reptiles [27, 28].

Current knowledge of exotic animal parasites is mostly based on cross-sectional surveys. Considering the continuous species alteration and the unidentified sources of the pets in the market, periodical surveys on the common pet species and their parasitic infection are inevitable. The wild-captured exotic animals may harbor various infectious organisms or they may have acquired the infection during translocation or in captivity in unsupervised conditions. It should be pointed out that inappropriate husbandry, mismanagement, and poor nutrition can suppress the immune system and lead to clinical symptoms [29]. The infected hosts in the present study and many similar studies worldwide were clinically asymptomatic [16, 20, 30]. The presence of prohibited species during the study signifies the boundless lucrative business of exotic animals and challenges the veterinarians for providing accurate husbandry and management advices [31-32].

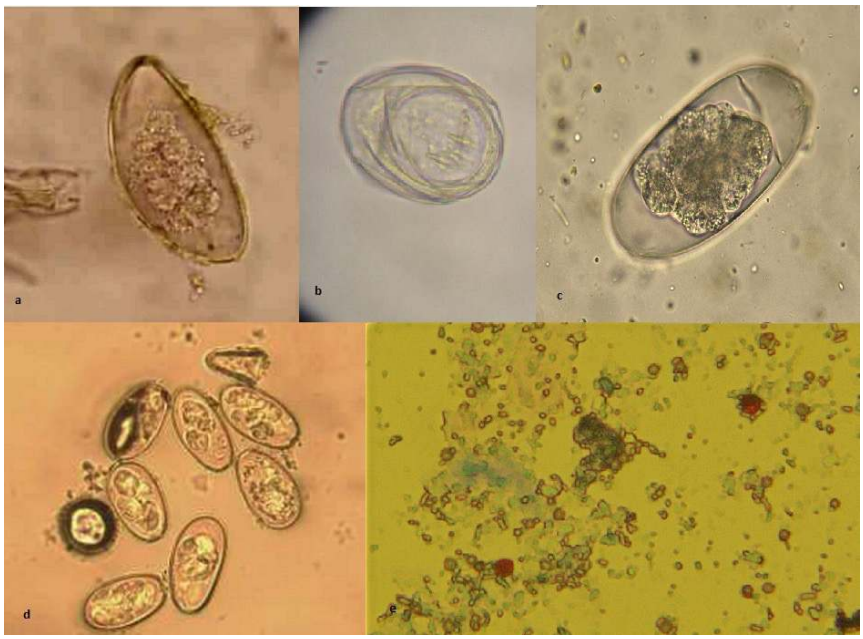

Figure 1.
a: Oxyurid eggs (*Passalurus ambiguus*) in rabbit feces. b: Cestode egg (note the 6-hooked oncosphere). c: Strongylid egg in rabbit feces (note the thin-shelled wall and the presence of the blastomeres. d: *Eimeria* sp. oocysts in rabbit feces. e: *Cryptosporidium* spp. oocysts in hamster feces (modified Ziehl-Neelsen stain, pink spherical organisms against the green background stain).

The well-being of the companion animal, in addition to the health of the client due to potential zoonotic pathogens should be considered [33-36]. Usually, there is no need for anti-parasitic therapy in an animal without clinical signs and infection. However, due to the close relationship of these animals with humans, especially with children and elderly owners, periodic parasitic monitoring tests are recommended.

Usually, exotic pet owners and breeders have insufficient information about the natural husbandry conditions. In sampling, it is essential to consider the intermittent excretion of parasites through defecation. In fact, with a single test, we may have false negative results. We recommend periodic sampling (three times at different intervals, depending on the type of parasite) to ensure the absence of Infections caused by parasitic diseases.. In this study, samples were collected and evaluated only once from each animal at the time of the hospital visit. Some studies using other diagnostic methods, such as post-mortem investigations (necropsy) or preparing slides directly from digestive tract cells, show different results. In the method applied in this study (flotation), saturated sodium chloride was used. One of the drawbacks of this solution is that it does not float heavy eggs such as *Trichuris* eggs. Also, to check the presence of amoeba and *Giardia*, it is adequate to mix the sediment sample with an iodine solution.

Materials and Methods

From July 2018 to March 2019, the fecal and dropping samples from referred exotic pets to the small animal Hospital of the Faculty of Veterinary Medicine, University of Tehran were collected. The samples were collected fresh in a single visit. For rodents, lagomorphs, and some reptiles, the process included pressing the rectal area or rubbing the cloacal area, for defecation stimulation. Occasionally, the samples were collected from the litter or the owners were provided with a container containing potassium dichromate 2.5% solution for sample collection.

Initially, the samples were investigated macroscopically with a stereomicroscope. A combination of direct wet smears, and smears after fecal flotation with saturated salt solution was performed on each sample [7]. Besides, modified Ziehl-Neelsen (MZN) staining was used for the detection of *Cryptosporidium* sp.

Statistical analysis using SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA) for computation of descriptive statistics and Chi-squared test to investigate the association between the presence and type of the parasites with host species and clinical symptoms ($p < 0.05$) was used.

Authors' Contributions

A. R., S. N. and F. A. conceived and planned the experiments. M.N. carried out the experiments. F. S. 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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Influences of Monosaccharides and Disaccharides Supplementations in Tris Media on the Motility Patterns of Fresh and Chilled Small Ruminant Spermatozoa

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ABSTRACT

In this study, the effects of monosaccharides, including glucose and fructose, and disaccharides, namely sucrose and trehalose, in eight Tris media on the motility patterns of small ruminants spermatozoa were investigated. Fresh and chilled semen samples from five Awassi rams and five Shami bucks were diluted in TBM and TEY containing 50 mM of the four different sugar types. The characteristics of spermatozoa motility were analyzed using a computer-assisted sperm analyzer (CASA). Fresh ram spermatozoa incubated in a TBM-fructose medium had the highest CASA values with no differences between the motility values generated from the fructose- and glucose-supplemented media. Trehalose reduced the values of velocity parameters, including VAP, VCL, and VSL for fresh ram sperm. Sucrose was the most influential sugar in raising the values of motility parameters MOT%, PMOT%, VAP, VCL, and VSL for fresh bucks spermatozoa, while trehalose generally had an important positive effect on chilled buck sperms. No significant differences ($p > 0.05$) were recorded for sperm trajectory parameters where the values of STR% and LIN% for the two ruminant species and the two spermatozoa types did not significantly differ between the eight media. It was concluded that during the first hours of in vitro incubation and based on the incubation temperature, the velocity parameters of small ruminant spermatozoa were the most affected CASA characteristics by monosaccharides and disaccharides supplementations in Tris semen media.

Keywords

Spermatozoa, Motility, CASA, Glucose, Fructose, Sucrose, Trehalose

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Abbreviations

AECS :Atomic Energy Commission of Syria
CASA: computer-assisted sperm analyzer
GLM: general linear model procedure
IVF: in vitro fertilization
LIN%: linearity percent
MDA: malondialdehyde
MOT% :motility percent
PMOT%: percent of sperm showing progressive motility
STR%: straightness percent
TBM: Tris-based media

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Introduction

It is well known that semen preservation media are needed to maintain spermatozoa viability and motility of the different animal species [1]. However, to sustain the motility status, spermatozoa require proper nutritional agents in the media. In this regard, sugar is one of the main constituents in semen media which can be easily metabolized into energy [2, 3]. Motility has been considered one of the most important indicators of sugar utilization by sperm because sugars provide the external energy source necessary for maintaining the motility status [2, 4]. It must be noted that when the environment does not provide any external energy source for the semen, the spermatozoa could use sugars in two ways: 1) obtaining energy by the Krebs cycle [5] and 2) storing sugars in the form of glycogen which presents a middle- to long-term energy reserve that could maintain motility [4].

Generally, sugars are divided into three major groups: monosaccharide, disaccharide, and trisaccharide. Monosaccharides are the main energy source for mammalian sperm [3], and fructose and glucose are the most important members of this sugar group. Fructose is an essential source of spermatozoa energy as it is metabolized and converted to pyruvate and lactate to support both sperm motility and viability [6]. In certain conditions, fructose acts as an extracellular cryoprotectant agent to protect the sperm membrane from toxicity during storage [7]. Glucose is also essential for energy utilization by spermatozoa [8]. In humans, glucose is required to sustain an optimal ATP concentration and to support optimum sperm motility [9]. Moreover, according to previous studies, glucose can support significant levels of hyperactivated motility and at the same time can be substituted by fructose.

Sucrose and trehalose are two disaccharides with the same molecular formula ($C_{12}H_{22}O_{11}$) but with different geometrical structures. Sucrose is composed of two monosaccharides, including glucose and fructose, while trehalose consists of two molecules of glucose. Sucrose is produced naturally in plants, from which table sugar is refined, while some bacteria, fungi, plants, and invertebrate animals synthesize trehalose as a source of energy. Sperm quality parameters of chilled and cryopreserved semen were shown to improve by using trehalose. The protective effects of this sugar significantly enhanced the freezability of buck and ram spermatozoa [10, 11, 12]. However, when trehalose was added in high concentration in a culture medium, sperm movement was hampered [13].

Sugar consumption by spermatozoa depends on both sugar type and sugar concentration. Matos-Brito et al. [14] showed that when extenders were used with appropriate concentrations of carbohydrates, goat sperm remained viable regardless of the initial concentration of fructose in goat seminal plasma. Moreover, Salamon and Ritar [15] noted that glucose and fructose addition to Tris buffer extenders resulted in higher post-thaw motility. Despite the importance of all the previously mentioned studies, the direct actions of sugars on spermatozoa are still little understood. In addition, to achieve the most efficacious use of fresh and chilled small ruminant semen, it is important to study the influence of diverse sugars on the motility status of these two spermatozoa types preserved in different semen media. Thus, the main objective of the present study was to assess the effects of using monosaccharides and disaccharides in Tris semen preservation media on the motility patterns of small ruminants spermatozoa, including fresh and chilled rams and bucks spermatozoa, during the first hours of *in vitro* incubation.

Result

Table 1 shows CASA motility values of fresh spermatozoa from rams incubated in TBM media supplemented with 50 mM of glucose, fructose, sucrose, and trehalose at 37°C for 60 min. The highest values of MOT% and PMOT%, as well as the velocity parameters VAP, VSL, and VCL were recorded when spermatozoa were incubated in the TBM solution containing fructose without recording any significant differences for MOT% and PMOT% between the four media. The addition of trehalose to the TBM solution led to a significant ($p < 0.05$) decrease in the values of VAP, VSL, and VCL in comparison with the spermatozoa incubated in TBM-sucrose, TBM-glucose, and TBM-fructose media. No significant differences ($p > 0.05$) were recorded between the four media containing monosaccharides and disaccharides for the trajectory parameters STR% and LIN%.

Table 2 shows CASA motility values of fresh spermatozoa from bucks incubated in TBM media supplemented with 50 mM of glucose, fructose, sucrose, and trehalose at 37°C for 60 min. The effect of sucrose was evident by increasing the values of MOT%, PMOT%, VAP, VSL, and VCL parameters in comparison with the other three sugar types. The clearest difference in motility values was between the medium containing sucrose and the base solution containing trehalose

Abbreviations-Cont'd

TEY: Tris-egg yolk
VAP: average path velocity VCL:curvilinear velocity
VSL: straight line velocity.

Table 1.
CASA sperm motion characteristics of fresh spermatozoa from rams incubated in Tris based medium (TBM) supplemented with 50 mM of glucose, fructose, sucrose and trehalose at 37°C for 60 minutes. Mean (± Sd) of CASA parameters: average path velocity (VAP), straight line velocity (VCL), curvilinear velocity (VCL), percent straightness (STR %), Percent linearity (LIN %) and the distribution percentage of motility subpopulation.

CASA Parameters	Glucose	Fructose	Sucrose	Trehalose
Motlity %	89.44 ± 2.92^{a}	93.5 ± 1.88^{a}	91.89 ± 2.26^{a}	89.55 ± 0.73^{a}
Progressive motility %	20.44 ± 1.33^{a}	22.56 ± 2.88^{a}	19.44 ± 1.42^{a}	18.66 ± 7.3^{a}
VAP (µm/s)	115.11 ± 4.01^{ab}	120.6 ± 11.9 ^{a}	114.89 ± 5.98^{ab}	109.33 ± 8.26^{b}
VSL (µm/s)	74.89 ± 4.04^{ab}	78.44 ± 9.25^{a}	69.67 ± 3.73^{ab}	67.89 ± 4.26^{b}
VCL (µm/s)	226.44 ± 7.32^{ab}	234.6 ± 11.4 ^{a}	221.44 ± 7.52^{ab}	210 ± 5.35^{b}
STR %	61.33 ± 2.65^{a}	59.88 ± 3.01^{a}	58.22 ± 1.79^{a}	60.44 ± 1.51^{a}
LIN %	33.22 ± 2.04^{a}	32.56 ± 2.3^{a}	30.33 ± 1.80^{a}	33 ± 1^{a}
Motility subpopulations				
Static %	11.22 ± 2.89^{a}	7.89 ± 3.05^{a}	8.89 ± 1.85^{a}	11.31 ± 1.73^{a}
Slow %	5.44 ± 2.89^{a}	4.55 ± 1.51^{a}	5.33 ± 1.69^{a}	10.48 ± 3.28^{b}
Medium %	23.77 ± 3.53^{a}	22.44 ± 2.52^{a}	26.22 ± 2.92^{a}	24.64 ± 1.92^{a}
Rapid %	59.55 ± 1.36^{ab}	65.11 ± 3.05^{a}	59.56 ± 1.37^{ab}	53.55 ± 1.37^{b}

The means (± Sd) with different letters (a-b) within columns significantly differ at $p < 0.05$.

Table 2.
CASA sperm motion characteristics of fresh spermatozoa from bucks incubated in Tris based medium (TBM) supplemented with 50 mM of glucose, fructose, sucrose and trehalose at 37 °C for 60 minutes. Mean (± Sd) of CASA parameters: average path velocity (VAP), straight line velocity (VCL), curvilinear velocity (VCL), percent straightness (STR %), Percent linearity (LIN %) and the distribution percentage of motility subpopulation.

CASA Parameters	Glucose	Fructose	Sucrose	Trehalose
Motlity %	87.67 ± 4.24 ^{ab}	89.6 ± 2.1^{ab}	92.77 ± 2.58 ^{a}	85.11± 3.98^{b}
Progressive motility %	20.77 ± 1.86^{b}	25 ± 1.73^{ab}	29.11 ± 1.61^{a}	21.11 ± 2.89^{b}
VAP (µm/s)	102.33 ± 5.34 ^{b}	114.4 ± 4.21^{a}	130.11± 8.79 ^{c}	107.66 ± 7.26^{b}
VSL (µm/s)	65.89 ± 2.26 ^{b}	75 ± 4.39^{a}	92.33 ± 7.78 ^{c}	71.33 ± 6.32 ^{a}
VCL (µm/s)	209 ± 5.12 ^{b}	215.6 ± 8.59^{a}	228.11± 12.25^{a}	211.22 ± 8.3^{ab}
STR %	58.66 ± 2.45^{a}	60.00 ±1.53^{a}	63.33 ± 1.23^{a}	60.77 ± 2. 6 ^{a}
LIN %	31.66 ± 1^{a}	34.11± 0.93 ^{a}	36.55 ± 2.06 ^{a}	34.77 ± 1.92 ^{a}
Motility subpopulations				
Static %	12.01 ± 3.46^{a}	11.66 ± 3.84 ^{a}	7.44 ± 2.13 ^{b}	14.77 ±3.11 ^{a}
Slow %	5.66 ± 1.72^{a}	4.33 ± 0.77 ^{a}	3.88 ± 0.38^{a}	3.11 ± 0.86 ^{a}
Medium %	14.01 ± 2.11^{a}	11.11 ± 0.96 ^{a}	9 ± 1.61^{b}	12.88 ± 3.57^{a}
Rapid %	68.33 ± 2.31^{a}	73.11 ± 3.07 ^{a}	79.77 ± 2.94^{b}	69.33 ± 4.35^{a}

The means (± Sd) with different letters (a-b) within columns significantly differ at $p < 0.05$.

(29.11 vs. 21.11 for PMOT%, and 130.11 vs. 107.66 for VAP, respectively), while no significant differences were recorded between the four TBM media for STR% and LIN%. Moreover, sucrose was also able to significantly raise ($p < 0.05$) the percentage of rapid sperm subpopulation compared to the other three media.

Table 3 shows CASA motility values of chilled spermatozoa from rams incubated in TEY media supplemented with 50 mM of glucose, fructose, sucrose, and trehalose at 5°C for 180 min. No differences were noted for none of the CASA motility characteristics between the chilled ram spermatozoa samples treated with different sugar types. Table 4 shows CASA motility values of chilled spermatozoa from bucks incubated in TEY media supplemented with 50 mM of glucose, fructose, sucrose, and trehalose at 5°C for 180 min. No significant differences ($p > 0.05$) were recorded for sperm incubated within TEY media containing the four types of supplemented sugars for both MOT% and PMOT% parameters. An increase in VAP, VCL, and VSL values was observed for the spermatozoa incubated in TEY medium containing trehalose, while there were no significant differences ($p > 0.05$) in the values of STR% and LIN % between the four different TEY media. Moreover, compared to the incubated spermatozoa in the TEY-fructose medium, a rise in the percentage of the rapid subpopulation category was caused by trehalose supplementation, and a decrease in the percentage of static sperm was recorded in this medium.

Discussion

Our present study is the first investigation that simultaneously shows the direct effects of monosaccharides and disaccharides on the spermatozoa motility pattern of two small ruminant species, including sheep and goat, and two spermatozoa types, including fresh and chilled samples. It must be stressed that motility is the main function of sperm cells and the main aim of energy obtainment. In this respect, sugar can be easily changed into energy, and the use of sugars, such as fructose, glucose, sucrose, and trehalose in semen media could increase sperm motility. It is well known that spermatozoa is a strict, glycolytic cell. Furthermore, the predominant metabolic pathways through which spermatozoa produce ATP, necessary for sperm motility, are mitochondrial oxidative phosphorylation and glycolysis [9]. In sperm cells, sugar, and especially glucose, is the main substrate for glycolysis, where it is metabolized to pyruvate and/or lactate to obtain cellular energy in the form of ATP. Generally, the effects of sugars may largely vary between species due to the differences in the chemical and physical composition of the sperm [16, 8]. Moreover, the differences in spermatozoa motility status may be due to several factors, including individual

Table 3.
CASA sperm motion characteristics of chilled spermatozoa from rams incubated in Tris egg-yolk medium (TEY) supplemented with 50 mM of glucose, fructose, sucrose and trehalose at 5 °C for 180 minutes. Mean (± Sd) of CASA parameters: average path velocity (VAP), straight line velocity (VCL), curvilinear velocity (VCL), percent straightness (STR %), Percent linearity (LIN %) and the distribution percentage of motility subpopulation.

CASA Parameters	Glucose	**Fructose**	**Sucrose**	**Trehalose**
Motlity %	91.55 ± 2.3[a]	91.11 ± 1.96[a]	92.67 ± 2.5[a]	91.56 ± 1.94[a]
Progressive motility %	16.55 ± 2.45[a]	16.44 ± 2.24[a]	16.11 ± 3.01[a]	15.11 ± 2.52 [a]
VAP (µm/s)	105.55 ± 7.23[a]	107.3 ± 6.42[a]	100.77 ± 4.52 [a]	99.11± 3.88 [a]
VSL (µm/s)	67 ± 5.12[a]	67.33 ± 4.58[a]	65.89 ± 4.25[a]	64.89 ± 4.98[a]
VCL (µm/s)	205.44 ± 8.35[a]	207.11 ± 6[a]	203.55 ± 4.36 [a]	199 ± 8.1 [a]
STR %	58.88 ± 1.76 [a]	59.33 ± 1.73[a]	58.77 ± 1.72[a]	60 ± 2.4 [a]
LIN %	31.33 ± 1[a]	31.78 ± 1.30[a]	31.55 ± 1.9[a]	32 ± 2.18 [a]
		Motility subpopulations		
Static %	9.77 ± 1.71[a]	9.11 ± 1.39[a]	8 ± 1.89[a]	9.78 ± 2.58[a]
Slow %	6.22 ± 1.84[a]	7.55 ± 2.04[a]	6.88 ± 1.66[a]	8.22 ± 2.71[a]
Medium %	29.44 ± 2.93[a]	27.77 ± 2.15[a]	29.88 ± 1.40[a]	29.66 ± 1.24 [a]
Rapid %	54.55 ± 4.72[a]	55.55 ± 2.77[a]	55 ± 1.59[a]	52.11 ± 2.57[a]

Table 4.
CASA sperm motion characteristics of chilled spermatozoa from bucks incubated in Tris egg-yolk medium (TEY) supplemented with 50 mM of glucose, fructose, sucrose and trehalose at 5 °C for 180 minutes. Mean (± Sd) of CASA parameters: average path velocity (VAP), straight line velocity (VCL), curvilinear velocity (VCL), percent straightness (STR %), Percent linearity (LIN %) and the distribution percentage of motility subpopulation.

CASA Parameters	Glucose	Fructose	Sucrose	Trehalose
Motlity %	90.77 ± 3.15[a]	90.44 ± 2[a]	89.77 ± 1.56 [a]	93.22 ± 2.28[a]
Progressive motility %	20.11± 2.82[a]	20 ± 2.7 [a]	19.44 ±1.23 [a]	22.11 ± 2.57[a]
VAP (µm/s)	86.56 ±9.14[b]	87.8± 5.31[ab]	92.22 ± 4.9 [ab]	96.77 ± 5.26 [a]
VSL (µm/s)	60.22 ± 6.30[a]	60.44 ± 3.17[a]	62.44 ± 2.9 [ab]	65.89 ± 5.13 [b]
VCL (µm/s)	184.66 ±10.16[a]	186.4 ± 9.72[a]	189.22 ± 7.07[a]	197.22 ± 4.57 [b]
STR %	61.66 ± 1.5 [a]	61.11± 2.93[a]	60.44 ± 0.72[a]	61.78 ± 2.28[a]
LIN %	32.78 ± 2.39 [a]	32.44 ± 1. 9[a]	32.22 ±1.09[a]	32.77 ± 1.72[a]
Motility subpopulations				
Static %	10.78 ± 2.38 [a]	10.33 ± 0.96 [a]	11.11 ± 1.36 [a]	8 ± 0.91[b]
Slow %	8.77 ± 1.94 [a]	8.44 ± 1.19 [a]	8.55 ± 1.59[a]	6.78 ±1.03[a]
Medium %	21.78 ± 1.93[a]	22.55 ± 1.72 [a]	21 ± 2.73[a]	20.88 ±3.29[a]
Rapid %	58.66 ± 2.80[ab]	58.22 ± 2.10 [a]	59.44 ± 2.66[ab]	64.33 ±3.70[b]

The means (± Sd) with different letters (a-b) within columns significantly differ at $p < 0.05$.

variation [17, 18], storage temperature [19], type of media [20], and also the differences in motility estimation methods (subjective or objective).

As sperm cells are dependent on their storage medium, different semen media, such as TBM and TEY, were developed to be used in assisted reproductive technologies. In this respect, sugar concentration is one of the most important points that should always be carefully considered during the preparation of any semen media, especially TBM and TEY. In the present study, a concentration of 50 mM for each sugar type was adopted in TBM and TEY media, as one of the concentrations usually used in Tris media [21]. Better maintenance of canine spermatozoa motility was noted when increased amounts of sugars were added to the semen extender. In this respect, the supplementation of TEY extender with 70 mM of sugars had notable beneficial effects on chilled canine spermatozoa compared to 10 mM, and this concentration resulted in significantly higher values for the percent motility and VAP parameter over the experiment period [22]. Moreover, compared to the freshly pooled canine semen, the mean values of VAP, VSL, and VCL increased significantly, suggesting that sugars activate sperm velocity [22]. However, in the present case, any higher concentrations over the 50 mM level of monosaccharides or disaccharides may cause osmolarity to become too high and in such a situation, substantial osmotic damage could be produced. It is well known that sperm cells are sensitive to osmotic stress [21, 23]. However, spermatozoa can tolerate a moderate range of osmolarities without a reduction in fertility. In this study, the osmolarity of the eight Tris media was within the range of physiological values (300-330 mOsm/Kg), which is the physiological osmolarity of most physiological fluids. Therefore, the osmolarity levels in our Tris media are not expected to have any significant effect on the motility status of rams and bucks spermatozoa.

The effects of monosaccharides on the metabolism of freshly ejaculated spermatozoa are very important for motility status. In dogs, sperm metabolize fructose and glucose in separate pathways resulting in separate systems of energy management indicated by different motility patterns and different roles in glycogen metabolism [2, 4]. The activating role of fructose and glucose on dog sperm was initiated by the intense and rapid increase in the tyrosine phosphorylation of some specific proteins [2]. Sperm functions could be modulated and modified immediately after ejaculation. However, further research in protein phosphorylation in the spermatozoa of small ruminant species treated by different sugar types must be conducted in the future.

On the other hand, is it possible to make a preference for a specific type of monosaccharides to be used in semen solutions? In general, the sperm of many mammals use glucose in preference to fructose when both substrates are available [24, 25]. Rogers and Perreault [26] observed that glucose supported better progressive human sperm motility than fructose, while Williams and Ford [9] found both sugars to be equally effective. In contrast, fructose has been found beneficial for frozen-thawed sperm in bovine [27] and ram [28]. It must be noted that in goat seminal plasma, fructose was the primary substrate for glycolysis [29] and the end product of the glycolysis pathway produces more ATP, necessary to support sperm motility. However, the combination of glucose and fructose as a supplement in the TALP medium improved the progressive motility of boar sperms [30]. In our study and during the first hours of in vitro incubation, we did not notice any clear differences between glucose and fructose, especially for the fresh and chilled ram spermatozoa. In agreement with these results, the survival of unfrozen ram sperms did not improve when ten different sugars were added [20]. Moreover, no effect was noted for sugar type (i.e., glucose, fructose, sucrose, lactose, and trehalose) on the motility of the post-thaw ram spermatozoa [31]. It must be pointed out that the monosaccharide metabolization pathways in mammalian spermatozoa depend on several factors, such as the medium composition, energy consumption rhythm, and most importantly the studied species where the sperm come from [3].

Diverse sperm types, semen media, and sugar groups used for supplementation could result in different motility patterns. In this study and unlike the motility status in the case of fresh spermatozoa types, trehalose had positive effects on CASA velocity parameters and the rapid subpopulation category of chilled goat spermatozoa. In contrast to our findings concerning chilled ram spermatozoa, the results of Zhao et al. [12] indicated that the addition of trehalose to Tris diluent improved the quality of long-preserved ram semen under low-temperature conditions. According to the previous authors, trehalose addition to Tris-fructose egg yolk medium at a concentration of less than 20 mM did not directly increase the progressive motility of sperm but significantly raised the integrity of the preserved sperm acrosome. In humans, superior post-thaw sperm parameters were observed by using 50 mM trehalose over sucrose and other trehalose concentrations [32]. It must be noted that trehalose exerts an indirect antioxidant effect by augmenting the level of glutathione and reducing lipid peroxide [33]. Chhillar et al. [34] documented that trehalose decreased H_2O_2 and MDA in frozen-thawed bull semen to the levels of fresh semen, while Badr et al. [35] reported similar results in buffalo. However, the antioxidant potential of trehalose on the spermatozoa of small ruminants and its relation to sperm motility pattern needs further research.

In the present study, we focused on fresh and chilled spermatozoa types. It must be pointed out that these two types could be used in different assisted reproductive technologies and they present a very important option for using cryopreserved ones. As the cryoprotective effects of monosaccharides and disaccharides on spermatozoa were well documented in the literature [36, 37], our present study was more focused on the motility aspect. However, the main result demonstrated that regardless of temperature, both monosaccharides and disaccharides could support the motility of bucks and rams spermatozoa during the first three hours of in vitro incubation in the Tris media.

Finally, two major limitations could be noted for the current study. The first is that sperm CASA motility parameters may not accurately predict the in vivo fertility results or IVF outcome. Therefore, other assessments of semen quality, including sperm viability, membrane, and acrosome integrity are needed. The second limitation is that motility status in this study was assessed during the first hours of in vitro incubation. In fact, we were interested in these particular incubation time points for both fresh and chilled spermatozoa samples because they represent the time window by which spermatozoa of such types could be directly used in different assisted reproduction technologies, especially AI. However, during the first three hours of in vitro incubation, our initial data clearly showed that the four sugars only affected the velocity, and not the spermatozoa trajectory of the small ruminants.

Conclusion

Taken together and by using CASA technology, this research showed the direct effects of monosaccharides and disaccharides supplementations in different Tris media on the spermatozoa motility patterns of small ruminants. It was clear that the sperm motility parameters of both rams and bucks were affected by monosaccharides and disaccharides based on the incubation temperature. Moreover, CASA velocity parameters were the most affected motility characteristics by sugars, while no differences were recorded for the sperm trajectory parameters. Such data could assist in the selection of the most appropriate sugar compatible with the temperature of keeping small ruminant spermatozoa within the semen media to achieve the best motility status. However, further studies are needed to test the in vivo and in vitro fertilization of small ruminants' spermatozoa incubated in different

media supplemented with different sugars.

Materials and Methods

Animals and ethical approval

This study was carried out at Der-Al-Hajar Animal Production Research Station, 33 km southeast of Damascus. Semen was obtained from five adult Awassi rams and five adult Shami bucks (two local Syrian small ruminant species). The animals aged 3-4 years, and were fed a diet based on concentrate, wheat straw, and barley, with water available ad libilum. It must be noted that the experiments for this study were approved by the Local Scientific and Ethical Committee of the AECS, Damascus, Syria (permit number 36-Z/M4 - 2019).

Media preparation, semen collection, and experimental design

All chemicals were purchased from Roth (Carl Roth Gmbh-Karlsruhe-Germany). Eight Tris media were prepared in the present study, including four TBM and four TEY. TBM prepared as a 300 mOsmol/Kg solution contained 200 mM tris (hydroxymethyl) aminomethane, 64.7 mM citric acid monohydrate, and 50 mM of each sugar type (glucose, fructose, sucrose, and trehalose), which were separately added to each base solution. For the TEY media, 20% of egg yolk was added to the four tris-based solutions with conserving the same previous concentrations of tris (200 mM), citric acid (64.7 mM), and the four sugar types (50 mM for each) in each TEY medium. It must be noted that the eight media were always held constant at pH 7.

In this study, a total of 60 ejaculates were collected from ten experimental animals (30 ejaculates from rams and 30 from bucks). Semen samples were collected using an electro-ejaculator (Minitube-Electro Ejaculator, Tiefenbach, Germany). Upon collection, semen specimens were immediately evaluated for general appearance and volume. For each animal and after semen collection, spermatozoa concentration was estimated using a haematocytometer (Neubauer Improved Marienfeld, Germany). An initial analysis of sperm motility was performed using the CASA system (Hamilton Thorne Biosciences, Version 12 CEROS, Beverly, USA). Sperm samples with a motility score ≥ 75% of motile spermatozoa and a concentration of ≥ 1×10^9 spermatozoa/ml were utilized. All ejaculations with no or poor motility status were immediately excluded before conducting the analyses. The collected ejaculates from each species were mixed in each replicate to isolate the individual effect of males. The fresh spermatozoa samples (25×10^6/ml) of both bucks or rams were incubated in TBM containing the four sugar types at 37°C in a water bath for 60 min, while the chilled spermatozoa samples (25×10^6/ml) of bucks or rams were incubated in TEY media containing the four sugar types at 5°C in the refrigerator for 3 h. Each experiment for each animal species and each semen media was repeated three times.

Motility assessment

The motility characteristics of the spermatozoa were assessed by CASA, using the Hamilton-Thorne motility analyzer (Hamilton Thorne Biosciences, Version 12 CEROS, Beverly, USA). For each sperm sample, three fields were selected, counted randomly, and assessed to generate data from at least 200-250 sperm/samples. The CASA characteristics included in the analysis were the MOT%, VCL (µm/s), VAP (µm/s), VSL (µm/s), LIN%, STR%, and PMOT% (VAP ≥ 75 µm/s and STR ≥ 80 %; HTM-CEROS; installation getting started guide version 12 CEROS). Spermatozoa subpopulations were defined in four categories by CASA as Rapid (4): fraction of all cells moving with VAP = 25 µm/s; Medium (3): fraction of all cells moving with 5 µm/s < VAP ≤ 25 µm/s; Slow (2): fraction of all cells moving with VAP < (5 µm/s or VSL < 11 µm/s; and Static (0-1): fraction of all cells not moving at all.

The Hamilton-Thorne motility analyzer settings used for goat spermatozoa were negative phase contrast optics at a recording rate of 60 frame/s, temperature of analysis 37°C, light adjustment 90-110, minimum cell size 5 pixels, non-motile head size 10 pixels, non-motile head intensity 80, low VAP cut off 20 µm/s, low VSL cut off 5 µm/s, static size limit 0.60/4.32 (min/max), and static intensity limit 0.20/1.92 (min/max). While the Hamilton-Thorne motility analyzer settings used for sheep spermatozoa were negative phase contrast optics at a recording rate of 60 frame/s, temperature of analysis 37°C, light adjustment 90-110, minimum cell size 5 pixels, non-motile head size 10 pixels, non-motile head intensity 80, low VAP cut off 21.9 µm/s, low VSL cut off 6 µm/s, static size limit 0.60/8 (min/max), and static intensity limit 0.25/1.50 (min/max).

Statistical analysis

Statistical analysis was conducted using the Minitab program (Minitab Coventry, United Kingdom, Version 13.31, 2000). Motility data were subjected to a factorial analysis of variance (ANOVA) for the four sugar types by GLM, followed by multiple pairwise comparisons using the Tukey posthoc test. The threshold of signification was set at $p < 0.05$.

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Competing Interests

The author declare that there is no conflict of interest.

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RESEARCH ARTICLE

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Therapeutic Intervention for Caseous Lymphadenitis Using Intra-abscess Instillation of Ozone or Hydrogen Peroxide in Small Ruminants

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ABSTRACT

CLA is an economically and zoonotically important disease in the world. The lack of a therapeutic procedure limits the treatment mainly to surgical intervention. Therapeutic efficacies of the intra-abscess instillation of O_3-oil and H_2O_2-gly in CLA in small ruminants were tested. One hundred eighty affected sheep and goats were allocated to five groups as follows: 1) NC (no intervention), 2) PC1 (injection of olive oil), 3) PC2 (injection of glycerin), 4) injection of O_3-oil, 5) injection of H_2O_2-gly. Samples of abscess contents were collected for microbiological examination prior to injection. The VAs were measured on T0, then with two-week intervals on T1 and T2. On T0 and T2, VAs were as NC (2.9 ± 0.5; 3.5 ± 0.5), PC1 (3.4 ± 0.7; 6.6 ± 1), PC2 (3.1 ± 0.7; 3.3 ± 0.9), O_3-oil (3.3 ± 0.4; 0.4 ± 0.4), and H_2O_2-gly (4.6 ± 0.4, 1.5 ± 0.4). Statistical analysis showed a significant decrease in VAs, merely in treatment groups. CP was recovered in 48.3% of bacteriological samples. The results of this study suggested that O_3-oil and H_2O_2-gly would be reliable therapeutic agents for treating and controlling CLA. Ozone showed apparently a higher efficacy and caused more rapid shrinkage/recession of the abscesses, compared to hydrogen peroxide..

Keywords

Abscess, Caseous lymphadenitis, Ozone, Hydrogen peroxide, Small ruminants

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Abbreviations

CLA: Caseous lymphadenitis
CP: *Corynebacterium pseudotuberculosis*
H_2O_2: Hydrogen peroxide
O_3: Ozone
NC: Negative control
PC1: Positive control 1
PC2: Positive control 2
O_3-oil: Ozonated olive oil

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Introduction

The CLA of sheep and goats caused by CP is an important problem worldwide. The chronic and insidious nature of the infection makes the control of the disease difficult, leading to a high prevalence in many parts of the world. Moreover, it causes significant economic losses in small ruminant populations [1]. The pyogenic superficial abscesses due to CLA, especially in the head and neck areas of sheep and goats, should always be considered as a source of serious concern for human health, herd health management programs, and profitability [1]. Based on the report of the United States Department of Agriculture in 2012, the national wool, mohair, and milk production economic losses were valued at $9.39 million. Losses in milk, meat, and wool production caused by CLA may be associated with substantial economic impacts on both low- and high-income countries [2].

CLA along with four other economically important diseases of sheep and goats, namely ovine Johne's disease, Maedi-Visna, ovine pulmonary adenomatosis, and border disease, collectively have been classified as "iceberg diseases" [3]. The presence of animals with the subclinical form of infection (the most challenging type for both practitioners and owners) is a common feature of these diseases [4]. Generally, CLA is a chronic (multi)systemic infection caused by a pyogranulomatous response of the lymphoid system, which ultimately results in the enlargement and abscessation of superficial and sometimes internal lymph nodes [3, 5].

The leading cause of CLA, CP, inhabits the environment and has rarely been isolated from the skin, nasal orifices, and ear canals of apparently unaffected sheep [7,8]. The most documented route for infection is thought to be skin wounds formed during various daily procedures, including unsafe or contaminated needle puncture [9].

Ruptured superficial abscesses play a crucial role in the spread of infection, whereas for pulmonary abscesses, such a role has not yet been fully established [6]. The isolation and identification of causative micro-organism(s) is the gold standard for diagnosis. Purulent materials and exudates are the most common specimens used for diagnostic purposes [3].

Abbreviations-Cont'd

H_2O_2.gly: H_2O_2-glycerol
T0: Baseline before treatment
T1: Time 1
T2: Time 2
Vas: Volumes of abscesses
BCS: Body condition score
LSM: Least square mean
SEM: Standard error of the mean
FDA: U.S. Food and Drug Administration

Classically, the standard surgical approach, including clinical lancing to drain purulent materials, is common when the abscesses mature. In recent years, some new therapeutic alternatives, such as antimicrobial photodynamic therapy, H_2O_2, and biogenic nanoparticles have been attempted with some promising results in cancer tumors and semi-spherical masses in human medicine. However, the need for relatively sophisticated equipment prevents them from being applicable in the current large animal field settings [10-12]. It has been suggested that potent bio-oxidants, such as H_2O_2 and O_3, in biologically safe forms and doses would be suitable candidates for investigating potential therapeutic intervention effect(s) in resolving encapsulated abscesses. The unique physicochemical properties of these compounds render them an eureka with the potential to be applicable in various biomedical fields [13].

Trioxygen, or O_3 can be administered as a pure gas in aqueous solutions (ozonated saline) or ozonated vegetable oils during ozone therapy. Ozone has been administered successfully through parenteral (IV, IM, SC, and intralesional) and loco-regional (cutaneous, rectal, vaginal, nasal, and dental) or even whole-body routes in human and animal patients [14]. Bio-oxidative therapy as a viable therapeutic modality offers medical and veterinary practitioners the best opportunity to safely deal with numerous pathological conditions, which are less responsive to conventional treatments and/or often fail after prolonged, frustrating, and costly interventions [13, 15].

Considering the therapeutic potentials of O_3 and H_2O_2, it seems such agents would be active in situations, where antimicrobials fail to penetrate or remain active in purulent environments. These capabilities could enable them to potentially sterilize the internal environment of the abscesses. These materials might be good candidates to be investigated as new approaches to deal with CLA, targeting the destruction of the pyogenic membranes of the abscesses.

From a practical standpoint, O_3 is an unstable gas that cannot be stored and should be used at once because it has a half-life of 40 min at 20°C and 140 min at 0°C [14]. However, the oxidant activity of O_3 in ozonated vegetable oils will be extended up to one year at 25°C or two years in the refrigerator [16]. Hydrogen peroxide is commercially available as a topical antiseptic agent for disinfecting the skin and superficial lesions [17]. It has also been frequently used for its well-known properties for wound healing and radiosensitization in the management of soft tissue tumors [18]. Direct application of pure H_2O_2 to wounds and body cavities may potentially cause intra-arterial oxygen embolism [17] and local mild to severe pain at injection sites [19]. Sodium hyaluronate has been

used as a solvent to alleviate the potential side effects of H_2O_2 injections inside the tumors prior to radiotherapy [17,19, 20], as well as inactivating the anti-oxidative enzyme peroxidase in tumors [21]. Glycerin or glycerol have been used as solvents as well to prolong the release of H_2O_2 [22].

Attempts to expedite the maturation and subsequent drainage of the abscesses could be the hallmark to stop the further spreading of the disease. The internal abscesses may occur on some occasions, with no overt clinical signs, serving as a source of disease proliferation through the visceral form of CLA believed to be associated with clinical manifestations of the so-called "thin ewe syndrome".

Apart from the surgical intervention, as far as the authors know, few pure therapeutic interventions have been devoted to the treatment of the disease. A closed-system lavage of abscesses and the intralesional administration of tulathromycin have been suggested [23]. A combination of intramuscular injection of rifamycin and tetracycline has been reported to be effective in the resolution of the enlarged and ruptured peripheral lymph nodes discharging thick green pus [24].

Based on the current knowledge, we hypothesized that the direct intra-abscess administration of either O_3 or H_2O_2 might have a beneficial effect on the treatment of CLA. The hypothetical basis is the assumption that the intra-abscess injection of a chemical agent that preserves its activity in purulent contents might have a recessive effect by sterilizing the pyogenic membrane. The objective of this field study was to study the impact of injecting either ozonated olive oil or 0.5% w/v H_2O_2 into intact CLA abscesses compared to control groups.

Result

A total of 208 affected abscesses from 180 animals [156 sheep (86.7%) and 24 goats (13.3%)] were included in this study. The distribution by treatment and the mean volumes of instilled agents are given in Table 1.

The isolated bacterial cultures from 180 animals (208 abscesses) are presented separately according to the type of isolated bacteria in each experimental group in Table 2.

The affected lymph nodes are described separately based on the type of anatomical position in each experimental group in Table 3.

The volume of the abscesses at different time intervals (T0, T1, and T2) are given in Table 4.

Volumes of abscesses differed significantly between treatments at T0 with the largest abscesses in the H_2O_2-gly group. Statistically significant

Table 1.
Abscesses, animals and average volumes of instilled agents in mL by treatment

	NC	PC 1	PC 2	O_3-oil	H_2O_2-gly
Abscesses (animals)	28 (30)	19 (17)	17 (13)	74 (61)	70 (59)
Volumes instilled	-	2.79 ± 0.9	2.72 ± 2.1	4.36 ± 3.7	5± 4.6

Number of animals per treatment are given in parenthesis

Table 2.
Isolated bacteria in the abscesses in all experimental groups (208 abscesses)

groups	C. pseudotuberculosis*	Streptococcus sp	Trueperella (Arcanobacterium) sp	Staphylococcus aureus	Staphylococcus sp	Rhodococcus sp	Micrococcus sp	Actinobacillus sp	Bacillus sp	Yersinia pseudotuberculosis	Actinomyces sp	Dermatophilus congolensis	Nocardia sp	No Growth	Growth of several colony forming units were ob-	total
PC1	11	1	1	1	2	1	0	0	1	0	0	0	0	3	2	23
PC2	10	2	0	2	0	0	1	0	3	0	0	0	0	1	1	20
O_3-oil	23	13	12	7	6	6	3	1	0	1	1	1	0	0	0	74
H_2O_2-gly	43	6	5	3	2	3	2	3	0	1	1	0	1	0	0	70
Total	87	22	18	13	10	10	6	4	4	2	2	1	1	3	4	
Total (%)	48.3	12.2	10.0	7.2	5.5	5.5	3.3	2.2	2.2	1.1	1.1	0.55	0.55	1.4		

• *Corynebacterium pseudotuberculosis*

Table 3.
Lymph node disruption involved with caseous lymphadenitis

groups	R*. mandibular	L^.mandibular	R. parotid	L. parotid	R. Retropharingeal	L. Retropharingeal	R. prescapular	L. prescapular	R. supra mammary(scrotal)	L. supra mammary(scrotal)	R. prefemoral(subiliac)	L. prefemoral(subiliac)	R. popliteal	L. popliteal	total
NC	8	3	5	3	3	1	1	2	1	0	1	0	0	0	28
PC1	5	8	0	6	0	0	0	0	0	0	0	0	0	0	19
PC2	2	6	3	4	0	0	0	2	0	0	0	0	0	0	17
O_3-oil	13	13	20	12	7	4	0	2	1	1	0	0	1	0	74
H_2O_2-gly	21	13	9	9	9	6	1	0	1	0	0	1	0	0	70
Total	49	43	37	34	19	11	2	6	3	1	1	1	1	0	
Total (%)	23.6	20.7	17.8	16.3	9.1	5.3	1.0	2.8	1.4	0.5		0.5	0.5	0	

* Right, ^ Left

Table 4.
The volume of the abscesses at different times are presented as LSM ± SE (mm^3)

	T0	T1	T2	Within group differences
NC	2.9 ± 0.5 €	3.6 ± 0.5 ¥	3.5 ± 0.5 ∑µ	= 0.366
PC1	a 3.4 ± 0.7 ¥€	b 5.6 ± 0.7 €®	b 6.6 ± 1®	= 0.031
PC2	a 3.1 ± 0.7 €	a 4.3 ± 0.7 ¥®	a 3.3 ± 0.9 £µ	= 0.211
O_3-oil	a 3.3 ± 0.4 €	b 1.9 ± 0.4 £	c 0.4 ± 0.4 €	= 0.01
H_2O_2-gly	a 4.6 ± 0.4 ¥	a 3.7 ± 0.4 ¥	b 1.5 ± 0.4 ¥	≤ 0.0001
Between group significant differences	= 0.0387	= 0.0137	= 0.0176	

Different alphabetical letters within a line represent statistically significant differences between time points. ($p < 0.05$)
Different symbols within a column represent statistically significant differences between treatments for each sampling time ($p < 0.05$)

Table 5.
Test of fixed effects. The mean volume of the abscesses at T0, T1 and T© have been presented as LSM ± SE mm^3

Variables	Groups										P-values		
	H2O2-gly		O3-oil		PC1		PC2		NC		Group (G)	Time (T)	G × T
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE			
Abscess volume	3.3	0.1	1.9	0.24	5.2	0.47	3.6	0.45	3.3	0.3	< 0.0001	0.25	< 0.0001

Table 6.
Number of ruptured abscesses in experimental groups

	NC	PC 1	PC 2	O_3-oil	H_2O_2-gly
Ruptured Abscesses	0 (None)	14 (73.68%)	11 (64.71%)	2 (2.70%)	2 (2.86%)

decreases in abscess volume over time were apparent for both treatments but not for any control groups. The volume of the abscesses in control groups remained unchanged, if not larger. The volumes of abscesses in the NC and PC2 groups remained unchanged, while in PC1 got larger significantly ($p = 0.031$, Tables 4 and 5). Abscesses in O_3-oil showed significant volume reductions in the first two weeks after treatment. However, a significant reducing effect was only observed after four weeks for H_2O_2-gly. The different responses in treatment vs. control groups showed the therapeutic effects of O_3-oil and H_2O_2-gly on the CLA abscesses.

We observed that O_3-oil caused a significant decline in the abscess volume compared to H_2O_2-gly at T1 ($p = 0.0002$) and T2 ($p = 0.0288$). The reducing effect of O3-oil was significantly higher in T1, compared to H_2O_2 ($p = 0.0002$) and control groups (PC1 = 0.0001, PC2 = 0.0012, NC = 0.0069). Moreover, the volume of the abscesses was greater in PC1 than in NC ($p = 0.0206$). The instillation of olive oil alone increased the volume of the abscess at T1 ($p = 0.03$) and T2 ($p = 0.01$) compared to T0 in PC1. The volume of abscesses showed no significant differences in T1 and T2 compared to T0 in the NC ($p = 0.366$) and PC2 ($p = 0.211$) groups (Tables 4 and 5). It should be noted that the unequal sizes of experimental groups were due to the refusal of the owners to allocate additional animals to the control groups because of observing adverse effects causing obligatory premature termination of the experiment in the control groups. Moreover, the significantly larger abscesses in the H_2O_2-gly group should be accounted as a shortcoming of the study design due to the strict adhesion to the rule of Latin square in allocating animals to the groups, while ignoring the importance of the similarity of abscesses in terms of size at the starting point. The number of ruptured abscesses in each group is presented in Table 6. On weekly follow-ups, the owners reported no relapse of abscesses and the complete disappearance of abscesses treated by O_3-oil or H_2O_2–gly.

The effects of gender, species, breed, age, and sex were evaluated as fixed effects. No significant effects were detected ($p = 0.1457$).

Discussion

The most important finding of this study is that the intra-abscess infusion of H_2O_2 and O_3 resulted in a significant decrease in the volume of the abscesses caused by CLA within four weeks (Table 2) to the extent that a considerable shrinkage and/or complete recession occurred on the sixth week.

The surgically opened abscesses should be routinely flushed with disinfectants, such as 10% povidone-iodine or iodine tincture; however, the histotoxicity characteristics of iodine solutions disrupt the healing process of the wounds [34, 35]. Biogenic silver or gold nanoparticles have been used as a postsurgical treatment instead of iodine solutions in cases of CLA with promising results [10, 12]. Moreover, postsurgical administration of antimicrobials could have great value as a part of the preventive strategy against the environmental re-infection of wounds.

The proposed therapeutic procedures apart from surgical intervention are somewhat problematic and inconclusive. The pathophysiology of the disease includes the formation of a thick pyogranulomatous lesion following organism establishment, and the development of an "onion ring" appearance in a process of repeated necrosis of the lesion following pathogenic agent proliferation, which causes the reformation and progressive enlargement of the capsule. This pathologic encapsulation structure precludes the penetration of antibiotics to the pyogenic membrane of the abscess, where the causative agent(s) reside(s) [1].

Combinations of oxytetracycline plus rifamycin or erythromycin have been used for treating CLA [24]. These combinations require the extra-label usage of antimicrobials for long periods, and some of them (e.g., erythromycin) are not labeled for use in food animals. Therefore, these shortcomings restrict their effectiveness either for food animals or other specific species [36]. A combination of subcutaneous usage of procaine penicillin G after opening, draining, and flushing the abscesses and/or intralesional administration of tulathromycin following a closed system lavage has been reported. The procedure has the same problems of requiring extended withholding times and extra-label usage of antimicrobials [23]. Collectively, the usage of antibiotics in the treatment of CLA has not been advocated extensively by veterinarians.

An alternative procedure similar to the present

study is the intra-abscess instillation of formalin, which is advised by some veterinarians [36]. Formalin is carcinogenic and not approved by the FDA. Moreover, it requires waiting for the maturation of the abscess, when it gets softness determined by palpation [36]. The latter point is a restriction to mass medication of the affected animals. Although not approved by the FDA yet, O_3-oil and H_2O_2-gly can be instilled into the immature abscesses, which makes it possible to treat all the affected animals in the flock in a single operational setting. This procedure lacks either the adverse effects of iodine solutions or the need for the extra-label usage of antimicrobials.

The mechanisms of action of H_2O_2 (as a 3% solution) have been attributed to directly killing microorganisms, and indirectly reacting as a signaling molecule or second messenger that stimulates effector cells to respond [18]. It has been reported that mild oxidative stress imposed on biological components by O_3 has several beneficial outcomes when used intralesionally in humans [37], providing fast cleansing of wound surface from pyonecrotic masses, stimulating the formation of granulation tissue, and enhancing wound healing [38]. Ozone reacts with the double bonds of triglycerides in vegetable oils, resulting in the production of ozonides and peroxidic species, which exert antimicrobial activity [39]. It is deemed that incorporating O_3 or H_2O_2 in olive oil or glycerin, respectively, extends the effects of these agents that react instantly per se. Antibiotics are deactivated in an abscess fluid environment due to low pH and oxygen tension, high protein content, high bacterial count, deactivating enzymes, and sequestration of bacteria engulfed by leukocytes [40, 41]. The agents O_3-oil and H_2O_2-gly retain their activity in purulent environments.

Several vaccines have been marketed against CLA, with varying results [36, 42, 43]. It should be noted that commercial vaccines mainly activate humoral responses and have some points of strength. First, they can limit the spread of an infection rather than eliminate an established infection. In other words, they are not suitable for the cure of affected animals, but their primary benefit lies in preventing the colonization of infection in vaccinated animals if used before their exposure to the organism [36]. Second, their potential for reducing the incidence of external and internal CLA lesions has been shown experimentally [4, 44-47]. Other interventions, including O_3-oil and H_2O_2-gly, lack such an important advantage.

In the present study, CP, as the leading causative agent of CLA, was isolated from 87 (48.3%) subjects, followed by a variety of other microorganisms (Table 2). In three abattoir studies, CP was isolated from 12.6% of cases in Iran [48], 34.7% in Poland [49], and 43.7% in Brazil [50]. Other researchers reported that CP was isolated from 27.84% of abscesses from clinically ill sheep and goats and condemned internal abscesses during meat inspection in Saudi Arabia [51]. In Egypt, CP was isolated from 90.07% of clinically infected cases [52]. In contrast, the major isolated bacteria from CLA cases in Spain was *Staphylococcus aureus* subspecies *anerobius* (44.4%), followed by CP (26.3%) [53]. Almost invariably, various other microorganisms were isolated in different studies, which could partly explain the variable results of vaccination against CLA in different geographical locations.

Mandibular lymph nodes were primarily affected in the present study followed by parotids, retropharyngeals, prescapulars, supramammaries, prefemorals, and right popliteals (Table 3). These data suggest that the lymph nodes of the head area are mostly affected, which might be due to the cumulative effects of the feeding behavior with the harsh environment as well as low-quality available range roughages in the desert (e.g., *Tamarix* and *Haloxylon* shrubs). This finding is consistent with the findings of other studies (33, 54, 55). The parotid lymph nodes were affected with higher frequencies in goats in Ethiopia and Egypt [52, 56, 57], while in Spain retropharyngeals were the most affected lymph nodes [58]. It seems that the lymph nodes of the head region are most affected by CLA.

Conclusion

The results of the present study show that the instillation of O_3-oil or H_2O_2-gly provides a promising result for the treatment of CLA abscesses in an early period of abscess growth. The authors believe that such a therapeutic intervention is highly needed due to the fact that solely relying on the management strategies have been failing in preventing the spread of the disease [59], so far. The managerial considerations are essential in preventing the discharge of the purulent contents of the abscess into the environment. The formation and rupture of superficial abscesses result in the release of large numbers of bacteria into the environment [1], which plays a vital role in the spread of CLA [60]. The microorganism(s) is (are) able to survive in the environment for several weeks [4]. In the present study, the intra-abscess instillation of O_3-oil and H_2O_2-gly prevented the spread of purulent contents into the environment in addition to decreasing the size of the abscesses, especially when they are immature. Moreover, in contrast to antimicrobials, no withdrawal time should be considered for O_3-oil and H_2O_2–gly. Additionally, contrary to antimicrobials that would be deactivated in purulent secretions, O_3-oil and H_2O_2-gly retain their

activity in an abscess environment, lacking concerns regarding antimicrobial residues for public health.

Future research is warranted, for example, to combine the instillation of O_3-oil and/or H_2O_2–gly into the abscesses and vaccination strategy when the internal abscess is also a matter of concern and de-

Materials and Methods

Study approval and Ethical Considerations

Ethical approval of the current experiment was issued by the Committee for Animal Welfare at the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, IRAN, (approval 3/48948 session 556-28/11/1397).

Methodology

1. Study Design, Inclusion Criteria and Data Collection

This prospective clinical case-control study was conducted in the field in the lowlands of Zirkuh district with the centrality of Hajiabad, located in south Khorasan province, East of Iran, adjacent to the Afghanistan border, with a cold and semi-arid mountainous climate and an average annual rainfall of 150 mm. The reference coordinates of the study location based on the GCS system are: 33°36'18"N , 59°59'36"E. All the animals in this region, which were referred to the authors with CLA during September 2018 August 2019 were included in this study. Animals with suppurative superficial lymphadenitis were considered eligible for allocation to the study only if they did not have: 1) any apparent clinical sign(s) consistent with a systemic infection, or 2) a history of receiving any local and/or systemic antimicrobial therapy for at least two weeks before the start of the study. Moreover, animals that treated with antimicrobials during the study or those that were not available for examination during the entire study period were retrospectively excluded from the dataset. The population in the present study mainly consisted of a native goat breed and different sheep breeds, including Balochi, Kurdi, Afshari, Chinese and other crossbreds. The flocks were kept on traditional nomadic management, grazing mainly sparse ranges and receiving occasionally supplemented feedstuffs, including wheat straw and barley grain. Vitamin/trace mineral supplements were provided for the flocks by the owners.

In the present study, two stable slow-releasing combinations, including an emulsion containing ozonated olive oil and a combination of H_2O_2 with glycerin, were selected to be injected into the intact superficial CLA-abscesses in volumes proportional to the size of each abscess. Animals included in the study were assigned to one of five treatments as follows: 1) no therapeutic intervention (NC, N.28 abscesses, 28 animals), 2) injection of olive oil into the abscess (carrier substance of O_3, PC1, N.19 abscesses, 17 animals), 3) injection of glycerol into the abscess (carrier substance of H_2O_2 , PC2, N.17 abscesses, 13 animals), 4)injection of ozonated olive oil (O_3-oil, N. 74 abscesses, 61 animals), and 5) injection of H_2O_2-glycerol into the abscess (H_2O_2-gly, N.70 abscesses, 59 animals) (Table 1). A total of 208 lymph nodes were examined from 180 affected sheep and goats in pre-existing groups (some animals had more than one abscess), which were clinically evaluated in terms of general health status and vital signs. The animal characteristics such as species, age, sex, breed, and BCS were also recorded at this stage.

2. Group Assignment

The animals were randomly assigned to each group, irrespective of the anatomical locations, gender of the animals or the size of the affected lymph nodes. Allocation was completed according to a Latin square, one by one to different experimental groups in five affected flocks on different occasions. The primary agreement between the researchers and owners were to allocate animals to experimental groups equally. However, the owners refused to allocate more animals to control groups, when they observed the poor response of injecting olive oil and glycerol alone into the abscesses, leading to a comparably fewer allocations to these groups.

3. Preparation of the therapeutic agents

Glycerol (1,2,3-propanetriol) (pharmaceutical grade, Merck Co.) was considered the best choice for dissolving H_2O_2 (pharmaceutical grade, Merck Co.) in terms of viscosity, availability and price, which could effectively maintain intra-abscess H_2O_2 concentrations while avoiding irritating effect of a pure 3% w/v H_2O_2 solution.

For each patient assigned to H_2O_2-gly, approximately 1 m1 of 3% w/v H_2O_2 solution (147 mmol/L) was thoroughly mixed with 5 m1 of 1% w/v glycerol (1:5 ratio) immediately befor injection under a treatment protocol introduced by Japanese investigators (KORTUC II protocol) [17, 19, 22] to obtain a final 0.5% H_2O_2 in glycerol solution.

Olive oil was chosen as a solvent for a mixture of oxygen and O_3 gases, resulting in the ozonated olive oil. The product has a longer half-life, and is more stable and applicable in field settings than the original gaseous O_3. Ozonated oils, are considered well-tolerated and safe compounds (LD50 > 2000 mg/kg BW), and remain stable at room temperature for up to one year and in the refrigerator for up to two years, provided to be stored in dark glass bottles to avoid sunlight. These properties altogether have led to the commercialization of pharmaceutical products containing ozonated oils for a variety of purposes [17, 26-28]. Sterile injectable emulsion of ozonated olive oil was prepared from sterile raw materials and packed in a class B environment in 10 ml amber vials aseptically. The emulsion was prepared by mixing 0.35% W/V of ozonated olive oil with sterile water for injection and 2% W/V of Tween 80® as an emulsifier in a laboratory scale homogenator. The peroxide, acid and iodine index of ozonated olive oil were 2439 ± 13.3, 17.3 ± 0.06, and 0, respectively [United States Pharmacopeia and the National Formulary (USP 35 - NF 30), Rockville (MD): The United States Pharmacopeia Convention; 2012].

Formulation and preparation of an injectable sterile solution of H_2O_2 3% w/v and glycerol 1% w/v (1:5 ratio), as well as an injectable sterile emulsion containing ozone in olive oil were performed in the school of Pharmacy, Mashhad University of Medical Sciences (MUMS).

No side effects, including the signs of pain or restlessness were manifested by the animals following the instillation of H_2O_2-gly or O_3-oil.

4. Clinical Examination and Sample Collection

Rectal temperature, as well as respiratory and heart rates for each animal were measured, and the conjunctival, oral and vulvar (in females) mucous membranes were examined to assess the general health condition of the animals. In addition, BCS was judged on a 1-5 numerical scale [28]. Superficial abscesses were identified and the location was recorded for each individual. The three dimensions (length, width, and height) of each abscess were measured in millimeters using a manual analogue Vernier calliper (WOLFOX®,127 mm), after thorough washing and shaving. The abscess volume (almost ellipsoid in shape in mm^3) was extrapolated from the dimensions using the following equation: Abscess Volume= $\pi/6 \times L \times W \times H$ [20].

The abscesses measurements were conducted at the time of inclusion in the study before treatment (baseline, T0) and again after two and four weeks. A fourth examination was conducted in the sixth week after treatment; however, the majority of abscesses in the control groups had been ruptured, while most of the abscesses were recessed in the treatment groups. The statistical

analysis was limited to T0, T1, and T2 with intervals of 2 weeks. Further follow-ups were carried out by calling the owners weekly.

5. Experimental procedures

The exposed surface of the abscesses was thoroughly washed and aseptically prepared with povidone-iodine surgical scrub, followed by wiping with a 70% isopropyl alcohol, letting to dry. The abscesses were punctured using disposable 18 G × 1.5" needles armed with a 2.5 m1 syringes. The abscesses contents were aspirated, while the tip of the needle was located at the opposite side of the puncture site, presumably near the pyogenic membrane of the abscesses. Aspirated materials were immediately transferred to a sterile, tightly capped tube. Samples were sent forwarded to the laboratory following strict recommendations regarding the shipment of microbiological specimens [29].

Prepared experimental agents corresponding to the respective experimental groups were then injected through the needle still in place. The animals assigned to NC did not receive any instillation, while abscesses in PC1 and PC2 were instilled with olive oil and glycerol without active compounds, respectively. The animals assigned to the treatment groups (O_3-oil and H_2O_2-gly) received either ozonated olive oil or an emulsion containing H_2O_2 in glycerol into the abscesses, respectively. The instillation was continued until the abscesses were felt to be filled by palpation. Therefore, variable amounts of solutions were instilled into the abscesses, which were recorded for all of them.

6. Bacterial Isolation and Identification

Samples were streaked onto Columbia agar supplemented with 8% defibrinated sheep blood and MacConkey agar. Blood agar plates were subsequently incubated in pairs for 48 h at 37°C, one aerobically and the other microaerophilically (candle jar). The pure isolates were identified based on colony characteristics, hemolysis pattern, microscopic cell morphology, and biochemical tests, namely catalase, oxidase, O/F test, SIM, gelatin hydrolysis, urea hydrolysis, nitrate reduction, MR/VP test, CAMP or reverse CAMP test , and carbohydrate fermentation profiles [30-33].

7. Data analysis

The current study was conceived with four evaluation time points T0, T1, T2, and T3, where T0 was the baseline immediately prior to the administration of experimental treatments and each following time point was two weeks after the previous one. Statistical analysis was limited from T0 to T2, as too few values for T3 were available for a meaningful analysis.

A difficulty for analysis was that the data of ruptured abscesses were missed after rupture. Assuming that larger abscesses were more likely to rupture than smaller abscesses, a missing value at one of the time points due to a ruptur decreases the mean abscess volume of the corresponding treatment and it thereby results in a bias that becomes increasingly important when more abscesses rupture in one treatment. In an attempt to quantify this effect, each abscess was assigned a categorical value for the parameter "treatment failure". The value 0 was assigned to the each abscesses with a volume of 80% or smaller than that of T0 at T1 or T2 that was 80% or less of the volume determined at T0. The value 1 was assigned to an abscesses with a volume of at least 80% of T0 at T1 and T2, and the value 3 was assigned to theabscesses that ruptured between T0 and T2. The cut-off value was set arbitrarily. Too few animals with data in T3 were available to be included in the statistical analysis.

8. Statistical analysis

Unless stated, the results are expressed as LSM ± SEM or as a median and interquartile range for variables not meeting the assumption of normality. The statistical significance level was set at $p < 0.05$. Data were tested for normal distribution and homogeneity of variance using Proc UNIVARIATE, and abscess volumes were square root transformed to achieve normal distribution.

To analyze of the square root transformed key outcome variable "abscess volume" a repeated measures analysis of variance was conducted using PROC MIXED. The animal ID was considered subject; as subject to determine the fixed effects of treatment, time, as well as the interaction between treatment and time, with time as repeated factor on the abscess volume. Covariates included in the initial model were sex, breed, and species of study animals. The covariates that were not statistically significant were removed from the initial analysis to obtain the definitive model. The autoregressive1 covariance structure was chosen based on the lowest Akaike information criterion. Bonferroni-adjusted p-values were used to assess differences between treatments at specific sampling times and also differences between sampling times whenever the F test was statistically significant. Proc FREQ applied frequency analyses on categorical variables, such as "treatment failure" and "pathogen identified in the abcsess" .

For convenience and because no preliminary data were available to make reasonable assumptions on treatment outcomes, the sample size of the present study was not based on a power analysis but on a convenience sample size that was all animals eligible for inclusion presented during one year.

All analyses were conducted with SAS software (SAS 9.4, SAS Inst. Inc. Cary, NC).

Authors' Contributions

G.A.K: examination of the animals, sampling and writing the manuscript. R.M.: Performing microbiological culture and examination procedures. O.M.: Preparation and quality control of the injectable products . B.N.: Finding the eligible flocks, examination of the animals, sampling, extensive participation in the process of writing the manuscript, and leadership of the field operations. M.A.B.: Supervision and planning the bacterial culture and examination procedures. K.S.: Designing the study, planning the field operations, writing the manuscript. All the authors have critically reviewed the manuscript.

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Competing Interests

The authors have nothing to disclose.

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Investigation of bacterial contamination with *Klebsilla* and *E. coli* in the prepucal cavity of pubertal and adult age in caprine

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ABSTRACT

The herein research was carried out in order to identified the presence of bacteria inside prepuce cavity of male caprine in both mature and pubertal age with focusing on *Klebsilla* and *E coli* species. Eighty prepuce swabs (fifty form mature and thirty from pubertal age) before slaughtering and cultured on blood agar and nutrient agar, bacterial isolation were identified with biochemical teats and finally by PCR. The present study found a significant difference ($p < 0.01$) between the prepuce swabs from caprine mature age (64%) and pubertal age (40%). Six various microorganisms were detected in prepuce samples in mature age, while four types were isolated from pubertal age. Positive isolation swabs detected the presence of *Staphylococcus aurues, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus* faecalis in both ages. Proteus mirabilis and *Klebsiella pneumonia* was isolated from mature age only. Significant isolation ($p < 0.01$) was appear of Escherichia coli among all different bacterial types. This research deduce that there was that the presence of bacteria inside prepuce of male genital system in both mature and pubertal age and their where a balance between genital immunity and localization of these bacteria and any stress factor may be lead to infection with such microbes, more over the mature male had more bacterial types due to the male matting behavior, finally the *E. coli* normally found in prepuce cavity as a normal flora of both ages and the Klebsilla species also found in mature age as a non-specific bacterial types.

Keywords

Klebsiella, Escherichia coli, Prepucal cavity, Pubertal age, Adult age, Goat

Abbreviations

PCR: Polymerase Chain Reaction

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Introduction

There is normal flora inside the body cavities without causing any diseases in the normal environment [1]. The importance of studying the normal inhabitant microflora inside the prepucal cavity lies in their role during the weakness of genital immunity due to several factors that may lead to infections with these microbes [1]. Several studies showed that a healthy genital system contains normal bacterial flora without any interference with reproductive functions [2, 3]. However, reproductive organs might get infected with unspecific bacteria that lead to decreased fertility [4]. The male is considered to be one of the causes of semen contamination [5]. The bacterial contamination of prepuce leads to penis contamination which can infect the female reproductive system during natural mating [6]. One of the complications of artificial insemination was contamination due to contact between the penis prepuce and the glans penis [1]. Some researchers stated that uterine inflammation may result from external factors entering the uterus during natural mating or incorrect artificial insemination [7]. While bacterial contamination has an adverse effect on spermatozoa fertilization ability due to its direct toxic impact on spermatozoa or indirect metabolic bacterial activity that interferes with sperm viability during semen storage. Therefore, semen is among the cofactors that spread genital infection [1]. Several factors may cause uterine infection, such as the entrance of microorganisms into the uterus cavity during mating [7], or during the usage of polluted semen [8, 9]. Glans contamination with bacteria leads to the contamination of the penis and the transfer of these bacteria into the female genital system during mating [6], indicating that the semen has a role in spreading the genital system infection of both genders as a carrier [9]. All of these factors make the idea to study the effect of age of puberty and maturity on the bacterial contamination and focusing on *Klebsilla* and *E. coli* species of the goat buck genitalia. The recent study was designed in order to detect the presence of bacteria inside prepuce cavity of male caprine in both mature as normal breeding animal and pubertal age as compensatory for aged breeding males with focusing on *Klebsilla* as non-specific bacterial types and E coli as a normal flora species types. Several researchers have studied infection with *E. coli* without evaluating its role in male genital organs [10, 11, 12, 13].

Result

The Table (1) listed isolation and then identification of bacteria from mature and pubertal age of male goat. The higher percentage of isolation was detected in mature age as compared with pubertal age with a significant differences at ($p < 0.01$) (Table 1). Moreover, mature male goats (64%) and pubertal male goats (28%) were significantly ($p < 0.01$) different in terms of positive isolation percentage (Table 1).

In the present study, six types of bacteria were isolated from prepuce of male goats in mature age with significant differences between the percentage of different species ($p < 0.05$) (Table 2). Four types were isolated from pubertal age with p <0.05 significant differences (Table 2). A higher percentage in isolation was recorded for *E. coli* species in both mature and pubertal age 32% and 23.3%, respectively (Table 2). There was a significant difference ($p < 0.05$) between the two ages and the prevalence of this type of bacterium was significantly different ($p < 0.05$) from all other bacteria in both caprine male ages (Table 2). The next percentage was noticed in S. aureus species which was recorded to be 24% and 16.7% in mature and pubertal age, respectively (Table 2) with significant differences between the two ages (Table 2). K. pneumoniae was isolated from mature male caprine only (6%) (Table 2). Proteus mirabilis was also recorded in 4% of mature caprine (Table 2). Streptococcus faecalis was identified in 6% and 10% of mature and pubertal age, respectively (Table 2). Pseudomonas aeruginosa was also isolated from 4% of mature-age and 6.7% of pubertal-age goats (Table 2). S. faecalis and P. aeruginosa also showed significant differences ($p < 0.05$) between the two ages (Table 2).

Table 1.
The number of prepuce samples, positive and negative isolation in puberty and mature male goat

Animal age	Samples positive isolation		Samples negative isolation		Significance
	number	Percentage	number	Percentage	
Mature	32	64%	18	36%	*
Puberty	12	40%	18	60%	*
Significance		*		*	

* Significant differences at $p < 0.01$.

Table 2.
Types of isolated bacteria from cervical samples during different estrus phases of slaughtering cows.

Types of isolated bacteria	Mature males		Puberty males		Significance
	Positive	Percentage	Positive	Percentage	
Escherichia coli	16	32%	7	23.3%	*
Klebsiella pneumoniae	3	6%	-	0%	*
Pseudomonas aeruginosa	2	4%	2	6.7%	*
Staphylococcus aureus	12	24%	5	16.7%	*
Proteus mirabilis	2	4%	-	0%	*
Streptococcus faecalis	3	6%	3	10%	*
Total isolation	38	*	15	*	

Some of these swabs contain more than one bacterium so the number of total will be more than positive isolation numbers.
* Significant differences between percentage at $p < 0.01$.

Discussion

This herein data showed that the external parts of male genital organs of goats had contamination with many various bacteria; our result become agrees in this part with others workers [6]. This study indicated that there were 64% of mature male goat had positive bacterial prepuce swabs as compared with 28% of pubertal male. This fact indicated that the male genital system contain normal microflora had no effect on reproductive activity. This is agreeing with other studies [2, 3, 4]. Some authors said that the isolation of bacteria from male and the female was symmetric [14]. This recent study is similar to this statement. Normal flora bacterial types could be activated during stress factors and it causes diseases as a pathogenic type [15]. This part indicated the importance of studying the contents of bacteria before using of male for breeding. This study isolated six types of bacteria represent the high percentage of it the localization of *Escherichia coli* and *Staphylococcus* aureus in mature age, whereas four types were isolated in pubertal age. This is lower than that which isolated from Al-Delemi *et al.* [1], and higher than Zaid *et al.* [16] in male goat. Zaid and Al-Zubaidy [15] claimed that there was relationship between bacterial number and bacterial types and fertilization of sperms; while Marinov et al. [17] stated that the 2nd ejaculation had little bacterial number than 1st one. The using of artificial techniques will be decrease uterine infections [18]. Bacterial contamination plays an important harmful role on uterus cavity especially the uterine glands [19]. Natural service considered as an important factors of uterine infection [20]. The response of endometrium against inflammation was controls by antigens and physiological events [7], it was appear within 0.5-1 hour after matting [15] this response is important to clear uterine cavity from bacteria and dead spermatozoa [16]. Lateness of uterine elimination from bacteria, fluid and debris after matting may results from many causes as myometrial activity decrease or myometrial activity duration will changed or myometrial activity frequency interacted [21], endometrium changed of vessels [15], altered hormonal response [21] mucus discharge stopping [22].

The result of identification of *Klebsiella* pneumoniae partially agrees with Aziz et al. [2] who said the importance of this microorganism in fertility. The lower percentage of isolation of *Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis* and *Streptococcus faecalis* due to it was considered as non-specific bacterium in genital system, so this may be the causative of lower isolation of those bacteria in pubertal age which related to decreases of natural mating with female than mature male. This type of bacteria is isolated in our result. This is fit with the result of Al-Delemi et al. [1]. The recent work detected that the possibility of make mixed bacterial isolation from the same swabs, this is agree with Al-Delemi [5].

The *E. coli* isolated by Al-Delemi [5] after matting and describes it as a normal flora inside uterine cavity, its origin from digestive cavity and may transported into urogenital tract of the female [20]. This isolation of such bacteria is done in the recent study. Al-Zubaidi et al. [23] stated that the *E. coli* caused vaginitis and the intravaginal sponge do not change microflora of vagina. *Esherichia coli* and *Pseudomonus aeruginosa* maybe result during matting from semen contamination with feces [15], or it came from female after mating due to its origin from female genital sys-

tem [1]. This is the origin of the bacterial types in our study. The release of Staphylococcus aureus in normal male semen with large number [16], also it had no pathological effect although of its high release [15], so it can easily identify in high level before or after matting. This may be explaining the higher percentage of isolation of such bacteria in our study. Al-Badry *et al*., [6] stated that this type of bacterium largely release with semen of ruminants without any clinical infections. The isolation of Proteus mirabilis was firstly done in this study from external part of genital organs; this indicated the existence of such bacteria flora in male genitalia. The bacterium may cause inflammation of female genital tract [24] in the she-camel. The increase of bacterial isolation may be come from dam during pubertal periods that appear again during maturation period [15]. This type of bacteria needs to be studied more for its relation with genital infection of both male and female. The isolation of Pseudomonus aeruginosa after matting in the herein study was previously detected by Al-Delemi [5] as a normal flora in ruminants. Pseudomonus aeruginosa had no damage effect to the genital tract [2]. This bacteria is isolated in the herein study. The presence of this bacterium inside semen results in a low fertility in male, and there was a high correlation with sterility [16]. The isolation of *Streptococcus* faecalis in high percentage in our study may came from infertility or abortion of the dam [15]. The limitation of our study its need to study the effect of matting behavior and female genital effect on bacterial contamination of external part of male.

From above we concluded that the presence of bacteria inside prepuce of male genital system in both mature and pubertal age. There were a balance between genital immunity and localization of these bacteria and any stress factor may be lead to infection with these microbes. Moreover, the mature male had more bacterial types due to the male matting behavior. Finally the E. coli normally found as a normal flora in prepuce cavity of both ages and the Klebsilla species also founded as a non-specific bacterial type.

Materials and Methods

Animals and sample collection

Eighty swabs were taken from the prepuce of healthy male goats before slaughtering consisting of 50 samples from mature males (around 3 years) and 30 samples from puberty males (below 1 year). These swabs were taken from the prepuce cavity after disinfecting the prepuce orifice with ethyl alcohol (70%, France). Cotton swabs (Amies transport medium, China) were inserted inside the prepuce cavity and moved several times. Then, these samples were transported to a private laboratory.

Isolation of bacteria

Media used to isolate the bacteria included sheep blood agar, MacConkey agar, nutrient agar, eosin methylene blue agar, and brain heart infusion agar. The media were incubated under aerobic conditions at 37°C for 24 hours. This was done according to the "Bergey's Manual of Systematic Bacteriology" [25].

Bacterial Identification

For all types of bacteria, identification was completed according to the "Bergey's Manual of Systematic Bacteriology" [26] by culturing, biochemical tests, and morphological characteristics. The observed characteristics of colonies on agar surfaces include color, size, consistency, pigment production, and shape. Cellular morphology was assessed by gram stain under a microscope. The biochemical tests included catalase, oxidase, IMViC, TSI, coagulase, urease production, gelatin liquefication, and hemolysis.

Identification by PCR

PCR (PreciGenome, USA) was used to amplify Escherichia genus-specific gene 16S rRNA of E. coli. Primer pairs were used to identify the gene (F 5'-GACCTCGGTTTAGTTCACAGA-3' and R 5'-CACACGCTGACGCTGACCA-3'). A total of 20 µl reaction mixture consisted of 3 µl genomic DNA, 10 µl PCR master mix (Promega, USA), and 1 µl of two primers. The final volume was adjusted to 20 µl with 5 µl of nuclease-free water. Initial denaturation was at 95°C for 5 min, denaturation at 94°C for 45 sec, annealing at 55°C for 45 sec, and extension at 72°C for 60 sec. The final extension was completed at 72°C for 5 min, and the reaction was performed in 30 cycles. Electrophoresis (Cleaver, UK) was conducted in 2% agarose gel at 100 v for 30 min. Staining was performed with ethidium bromide under a UV trans-illuminator according to Schippa et al. [27]. For K. pneumonia, the detection was performed by inf B1 gene using the primers (F 5`-CTC-GCTGCTGGACTATAT TCG-`3 and R 5`-CGCTTTCAGCT-CAAGAACTTC-`3). A reaction mixture of 25 µl contained 2 µl DNA, 1 µl of two primers, 12.5 µl master mix, and 8.5 µl nuclease-free water. Initial denaturation was at 95°C for 5 min, 30 cycles at 95°C for 0.5 min, 55°C for 30 sec, 72°C for 0.5 min, and final extension at 72°C for 7 min. The PCR product was analyzed (PreciGenome USA) using gel electrophoresis (Cleaver UK) 1% agarose, was stained with ethidium bromide, and visualized with a UV illuminator. This was completed according to Abd Alwahed et al. [28].

Statistical analysis

Statistical analysis was performed using the Chi-square test to detect the variation between the percentages of groups at $p < 0.01$ and $p < 0.05$. This was performed using SAS [29].

Authors' Contributions

Ansam Khalid Mohammed conceived and planned the experiments and carried out the experiments with planned and carried out the simulations and contributed to sample preparation and contributed to the interpretation of the results and took the lead in writing the manuscript. The author 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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Sexual Dimorphism in Clinical Chemistry and Profile of Hybrid Catfish (*Heterobranchus longifilis*)

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ABSTRACT

Sex has been reported to influence the clinical chemistry of several species of fish. Whether sex impacts serum biochemistry composition and electrolyte profile of *Heterobranchus longifilis* is not well captured in the literature. This study aimed to evaluate the impact of sex on the clinical chemistry composition and electrolyte profile of hybrid catfish, *Heterobranchus longifilis*. Blood samples were collected and biochemically analyzed. The analytes analyzed included alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, creatinine, total bilirubin, conjugated bilirubin, unconjugated bilirubin, serum protein, albumin, total cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride, and urea. Uric acid, bicarbonate, Chloride, Sodium, and Potassium from 40 healthy *Heterobranchus longifilis* (20 males and 20 females). The males and females were not reproductively active at the time of sampling (None of the females had eggs). Most clinical chemistry analytes and electrolyte profiles of *Heterobranchus longifilis* showed differences between male and female values even though only a few (alanine aminotransferase, aspartate aminotransferase, creatinine, triglyceride, and uric acid) were statistically significant ($p < 0.05$). Based on the findings in this study, we suggest that sexual differences affect the clinical chemistry and electrolyte profile of Heterobranchus longifilis. Hence, sexual differences should be taken into consideration during sampling in both natural and experimental studies in Heterobranchus longifilis.

Keywords

Electrolytes, Heterobranchus longifilis, clinical chemistry, Sex

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Abbreviations

ALP: Alkaline phosphatase
AST: Aspartate aminotransferase
ALT: Alanine aminotransferase –
HDL: High-density lipoprotein – HDL
LDL: Low-density lipoprotein
CSB: Conjugated serum bilirubin
UCSB: Unconjugated serum bilirubin

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Introduction

Fishes are increasingly getting more recognition as an economic and reliable source of quality protein of animal origin owing to their rich nutritional values [1]. Aquaculture is presently one of the fastest-growing sectors of the food production industry globally, taking about 50 % of the overall food supply [2]. Aquaculture is known to be one of the most efficient means of food production [3, 1]. Fish is widely consumed by a range of people, notwithstanding their age, level of income, or health status [4, 2]. *Heterobranchus longifilis*, on the other hand, has been documented to have the capacity for an efficient feed conversion rate [5]. Several criteria made *H. longifilis* suitable for aquaculture, and these qualities include its capacity for high yield potential, fast growth rate, high fecundity, hardiness, and palatability [6]. Serum biochemistry analysis could help in identifying target organs of toxicity in addition to unraveling the general health status of fish [7, 8]. Several scholars have reported that the biochemical parameters of fish have become useful tools for the determination of physiological and pathological changes in diverse fish species [9, 10, 11]. This is because these biochemical indices offer valuable information on the responses of fish to diverse ecological and physiological changes [5, 12, 8]. Additionally, several pathological changes are reflectedin serum chemistry long before the manifestation of clinical diseases [13, 8].

The values of biochemical parameters are substantially influenced by several physiological factors, including the sex of the fish [14]. A study assessing serum chemistry parameters discovered that sex can induc some level of influence on some parameters of C. gariepinus [15]. Other scholars have repeatedly reported that sex, age, diet, fish species and strains, nutritional state, geographical location, disease, feeding regime, sexual maturity cycle, and seasonal variations in temperature and salinity, can strongly influence values of biochemical parameters and health status of fish [4, 5, 1, 11]. While several studies have evaluated the serum biochemistry of different species of fish, there is limited information on variations in serum chemistry and electrolyte profile of *H. longifilis* concerning sex. The influence of sexual differences on serum chemistry analytes and electrolyte profiles in H. longifilis may offer valuable baseline information that could enhance further studies on mechanisms associated with the influence of sexual differences on the biochemical parameters of fish. This study aimed to evaluate the impact of sexual dimorphism on the serum biochemical and electrolyte profile of hybrid catfish (*Heterobranchus longifilis*).

Result

Mean Serum enzymes

Analysis of the serum enzymes results showed mean serum Aspartate Aminotransferase (AST) level of male *H. longifilis* was higher with a mean value of 114.6 U/L compared to the females with mean serum AST value of 111.2 U/L while the mean AST value of both males and females combined was 112.9 U/L (Fig. 1). For ALP on the other hand, the mean serum Alkaline Phosphatase level of the male H. longifilis is not significantly different ($p > 0.05$), with a mean value of 37.8 U/L compared to the female with mean serum Alkaline Phosphatase value of 40.1 U/L while the mean Alanine Aminotransferase value of both male and female combined was 39.0 U/L. Even though there was no statistically significant difference between sex, the ALP value of the females unlike AST, was slightly higher compared to that of the males. The mean serum alanine Aminotransferase (ALT) level of the male H. longifilis was however, significantly higher ($p < 0.05$) with a mean value of 28.5 U/L compared to the females with a mean value of 18.5 U/L while the mean Alanine Aminotransferase value of both male and female combined was 23.5 U/L.

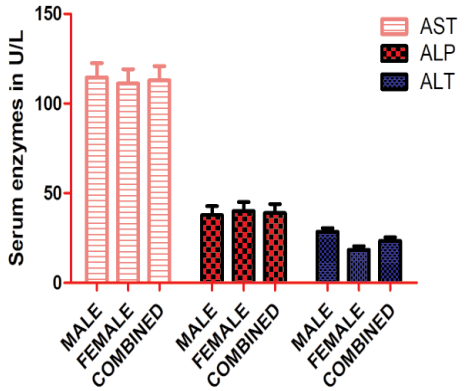

Figure 1.
Mean Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), and Alanine Aminotransferase (ALT) from male and female H. longifilis and combined values, all in mg/dL

Mean serum Creatinine level

Analysis of the serum creatinine level showed serum creatinine level of male *H. longifilis* was significantly higher ($p < 0.05$) compared to the females with mean serum creatinine values of 50.7 µmol/L as against 44.5 µmol/L while the mean creatinine value of both male and female combined was 47.6 µmol/L (Fig. 2).

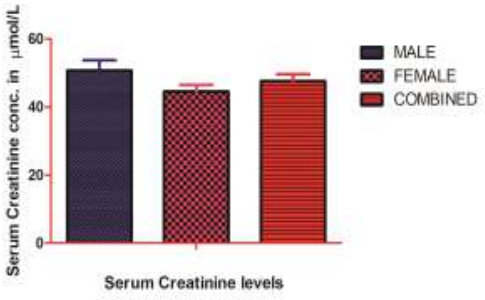

Figure 2.
Mean serum Creatinine concentration from male and female *H. longifilis* and combined mean serum Creatinine value

Serum Bilirubin levels

Analysis of the results showed there was no statistically significant difference between male and female *H. longifilis* in mean total serum bilirubin levels. The male and female mean total serum bilirubin values were 14.0 mg/dL and 13.9 mg/dL respectively while the mean value of both males and females combined was 14.0 mg/dL (Fig. 3). There was also no statistically significant difference between male and female H. longifilis concerning conjugated serum bilirubin (CSB) level, even though the males had substantially higher values compared to the females. The male and female mean CSB values were 8.23 mg/dL and 6.12 mg/dL respectively while the mean value of both males and females combined was 7.18 mg/dL. Statistical analysis of the unconjugated bilirubin results also showed there was similarly no statistically significant difference ($p > 0.05$) between male and female *H. longifilis*, even though the value was higher in females compared to males. The male, female, and combined mean UCSB values were 5.77 mg/dL, 7.78 mg/dL, and 6.77 mg/dL respectively.

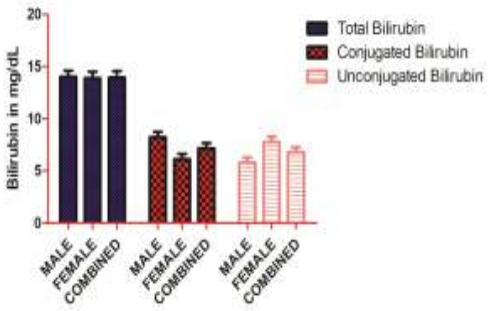

Figure 3.
Mean values of total, conjugated and unconjugated Bilirubin concentrations from male, and female *H. longifilis*, and their combined mean values

Serum Protein and Albumin Levels

Statistical analysis of protein and albumin results showed there was no statistically significant difference ($p > 0.05$) between male and female *H. longifilis* with regards to mean total serum protein level (Fig. 4), even though the value was substantially higher in females compared to males. The male and female mean total serum protein values were 53.0 g/dL and 61.0 g/dL respectively, while the mean value of both male and female combined is 57.0 g/dL. Analysis of the albumin results showed no statistically significant difference between male and female *H. longifilis*, even though the value was higher in males compared to females. The male, female, and combined mean serum albumin values were 24.5 g/dL, 26.2 g/dL, and 25.4 g/dL respectively.

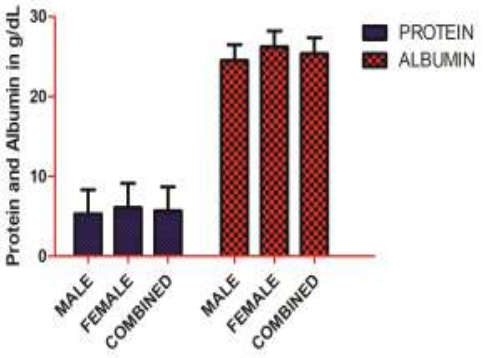

Figure 4.
Mean serum total Protein and Albumin concentrations from male and female *H. longifilis* and their combined mean values in g/dL.

Total serum Cholesterol level

Results of serum cholesterol, as observed in this study, are depicted in Fig. 5. Analysis of the results showed there was no statistically significant difference ($p > 0.05$) between male and female *H. longifilis*, even though the value was slightly higher in females compared to males. The male and female mean total serum cholesterol and their combined values were 2.42 g/dL, 2.62 g/dL, and 2.52 g/dL, respectively.

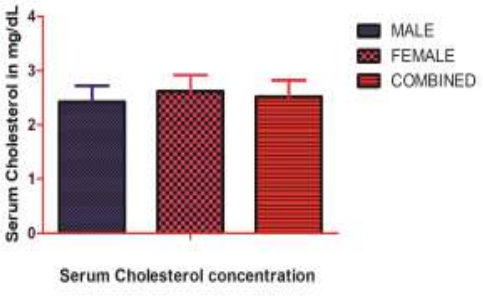

Figure 5.
Mean serum Cholesterol concentration from male and female H. longifilis and combined mean serum Cholesterol value

Mean Serum High-Density Lipoproteins and Low-Density Lipoproteins Levels

Statistical analysis of lipoproteins results showed there was no statistically significant difference ($p > 0.05$) between male and female *H. longifilis* in serum HDL levels (Fig. 6), even though the value was substantially higher in females compared to males. The males and females had mean serum HDL values of 1.35 mg/dL and 1.63 mg/dL, respectively while the mean value of both males and females combined was 1.49 mg/dL. Analysis of serum LDL results, on the other hand, showed that there was no statistically significant difference between male and female *H. longifilis,* even though the value was slightly higher in females compared to males. The male and female mean LDL values and the combined values were 1.20 mg/dL, 1.35 mg/dL, and 1.28 mg/dL, respectively.

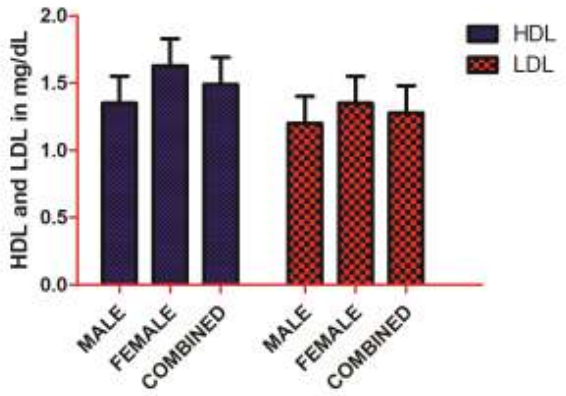

Figure 6.
Mean High Density and Low-Density Lipoprotein levels from male and female *H. longifilis* and their combined mean values

Mean Serum Triglyceride level

The results of serum triglyceride levels in males, and females and their combined value are depicted in Fig. 7. Analysis of the results showed there was a statistically significant difference ($p < 0.05$) between male and female *H. longifilis*, with the females having higher value (1.65 mg/dL) compared to the male with serum triglyceride values of 1.39 mg/dL, while the mean value of both male and female combined is 1.52 mg/dL.

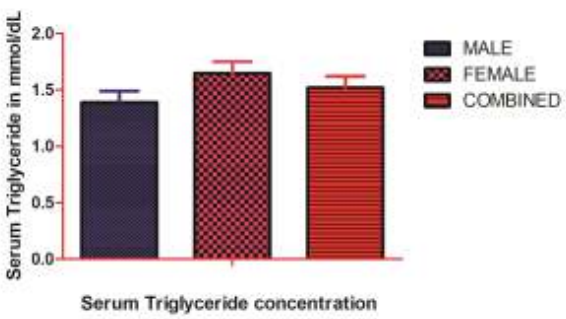

Figure 7.
Mean Triglyceride concentration from male and female *H. longifilis* and combined mean Triglyceride value

Mean Serum Urea and Uric acid Concentrations

Mean serum urea and uric acid concentrations for males, females, and their combined value are shown in Fig. 8. Analysis of the results showed there was no statistically significant difference ($p > 0.05$) between male and female *H. longifilis* in serum urea concentration, even though males had slightly higher serum urea concentration compared to the females. The male and female serum Urea concentrations were 3.20 mmol/L and 2.90 mmol/L respectively while the mean value of both male and female combined is 3.05 mmol/L. On the other hand, results showed a statistically significant difference ($p < 0.05$) between the concentration of serum uric acid in male and female *H. longifilis*. The male, female, and combined serum uric acid concentrations were 65.45 µmol/L, 71.87 µmol/L, and 68.66 µmol/L respectively.

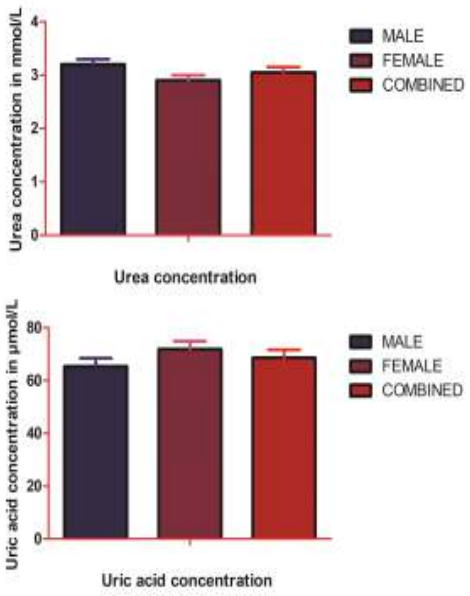

Figure 8.
Mean Urea and Uric acid concentrations from male and female *H. longifilis* and their combined mean values

Mean Serum Sodium and Chloride Concentration

Sodium and chloride concentrations for males, females and combined values are presented in Fig. 9. Statistical analysis of the results showed no statistical-

ly significant difference ($p > 0.05$) between male and female *H. longifilis* in serum Sodium concentration. The male and female serum sodium concentrations were 145.44 mmol/L and 139.12 mmol/L, respectively, while the mean value of both males and females combined was 142.28 mmol/L. Even though there was no statistically significant difference in Sodium concentrations between males and females, the mean sodium concentration of the males was slightly higher than that of the females. chloride results showed no statistically significant difference between male and female *H. longifilis*. The male, female and combined serum Chloride concentrations were 109.27 mmol/L, 102.0 mmol/L, and 105.64 mmol/L respectively. However, the mean Chloride concentration of the males was substantially higher when compared to that of the females.

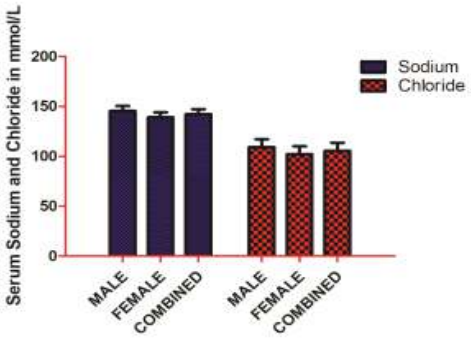

Figure 9.
Mean serum Sodium and Chloride concentrations from male and female *H. longifilis* and their combined mean values

Mean Serum Bicarbonate and Potassium Concentrations

Bicarbonate and potassium concentrations observed in this study for males, females and combined values are shown in Fig. 10. Statistical analysis of serum bicarbonate concentration results showed there was no statistically significant difference between male and female *H. longifilis*. The male and female serum bicarbonate concentrations were 27.55 mmol/L and 25.97 mmol/L respectively while the mean value of both male and female combined is 26.76 mmol/L. Although there was no statistically significant difference between serum bicarbonate concentrations of males and females, the mean serum Bicarbonate concentrations of males were slightly higher compared to that of the females. For serum potassium concentration, on the other hand, there was no statistically significant difference between male and female *H. longifilis*. The male, female, and combined serum potassium concentrations were 6.25 mmol/L, 5.10 mmol/L, and 5.68 mmol/L respectively. The mean potassium concentration of the males was higher compared to that of the females.

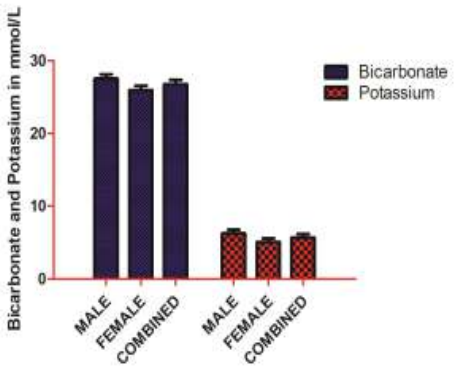

Figure 10.
Mean serum Bicarbonate and Potassium concentrations from male and female *H. longifilis* and their combined mean values

Discussion

Fish are the largest and most extensively diversified species of aquatic organisms. Owing to their proximity to the aquatic environment, alterations in their environment are rapidly expressed in their blood [16, 11]. Diverse scholars are increasingly interested in the study of clinical chemistry characteristics of fish as they reflect the overall health status of fish. These indices offer reliable insight into metabolic disorders, chronic conditions, and deficiencies, before clinical manifestations [12, 16]. Several pathologic changes have been reported in serum chemistry long before the manifestation of any clinical disease [12, 16]. In this study, the mean serum AST and ALT of the males were significantly higher compared to those of the females, while ALP was higher in females even though insignificantly compared to the males. The observed differences in serum enzymes between males and females are in tandem with the findings in other recent related studies, where different species of fish were found to have differences in levels of these serum enzymes and attributed the difference to sex-linked physiological processes in the fish [5, 14, 12]. However, while few scholars [17, 13] found that AST and ALT were higher in females than males, the contrary was our findings in this study. The differences may not be unconnected with several physiological factors and environmental conditions and many other factors such as temperature and quality of the water management practices at the time of such experiments, since fish are known to be in close proximity to their environment [11].

Creatinine is excreted through the kidney, hence

an increase in the level of serum creatinine in fish reflects severe kidney injury [14]. In this study, the mean serum creatinine levelwas higher in males than females, even though the differences were not statistically significant. These findings agreed with the results of earlier studies [17, 14], where male rainbow trout and Notopterus notopterus were reported to have higher creatinine levels than females. The overall creatinine levels in both males and females were not high, which indicates the normal function of the kidney in both males and females in this experiment. Bilirubin is a bile pigment synthesized endogenously, and its accumulation in the body could be toxic. An increase in the conjugated bilirubin concentration of serum reflects a distorted balance between the rate of haem conversion to bilirubin and the capacity of the liver to produce conjugated bilirubin [18, 19]. In this study, total bilirubin and conjugated bilirubin in males were slightly (statistically insignificant) higher than in females, while unconjugated bilirubin in females was significantly higher than in males. This study reported a higher level of unconjugated bilirubin in females, which is consistent with previous studies that female *H. longifilis* had higher bilirubin levels than males [20, 5].

Changes in total serum proteins and albumin are known to be clinically relevant in establishing the health status of fish [21]. The total serum protein level is a critical and reliable indicator to evaluate the physiological status, nutritional state, stress, and general well-being of fish [22]. The higher level of total serum protein observed in females compared to males confirms the findings reported by Sharma et al. [21] and Jan and Ahmed [10], who found higher total serum protein in snow salmon and Schizothorax labiatus and Barilius bendelisis, respectively. Similar to the findings of this study, other studies [23, 21] have reported higher albumin levels in Salmo trutta fario and beluga whale (Huso huso) males compared to females. However, the differences in albumin levels between males and females were not statistically significant, and this has also been similarly reported in a related study [10]. These differences in both total serum protein and serum albumin have been attributed to feed intake, starvation, growth rate, andfeed conversion rate [24, 25]. Total serum protein varies seasonally between sexes [10].

Generally, cholesterol is vital for appropriate body function as it serves as a substrate for the synthesis of several crucial and active biological constituents, including sex hormones [13, 21]. Several scholars have reported that seasons and rate of growth/stage influence cholesterol levels [5, 10]. In this study, there was no significant difference in cholesterol levels between males and females, even though that of the females was slightly higher compared to males. These results confirm the findings reported in related studies, where cholesterol levels did not significantly differ between males and females. [5, 21]. In fish, lipid storage serves as the primary energy reserve, and fluctuations in serum lipid levels in various fish species have been recorded [26, 21]. High-density lipoprotein cholesterol could be determined directly from the serum by enzymatic techniques, using cholesteryl esterase and cholesterol oxidase methods [27]. In this study, there was no significant difference in HDL between males and females, even though the HDL in females was slightly higher compared to females. In a related study, Sharma *et al.* [21] reported similar findings in male and female Barilius bendelisis from Central Himalaya, India. On the other hand, low-density lipoproteins are recognized as mediators of cholesterol and cholesterol ester absorption in several fish tissues [14]. The higher LDL levels in females compared to males, as observed in this study, were also reported in an earlier study, and the differences were attributed to reproduction, maturation, and metabolic rate of the fish [21].

The level of triglyceride, in concurrence with other lipids, is valuable in the diagnosis of several conditions, such as triglyceridemia, dyslipidemia and hyperlipoproteinemia [28]. The observed higher levels of triglyceride in females compared to males in this study are similar to the findings reported in an earlier related study [21], where female Tench (Tinca tinca) had higher levels of triglyceride compared to males. The variations in the triglyceride between males and females were attributed to different metabolic rates, feeding intensity, and seasons [29, 21]. The concentration of urea in the blood is a reflector of protein metabolism in the system [16]. In this study, the level of urea was higher in males compared to females, even though the difference was not statistically significant. Several researchers have also documented higher urea concentrations in males compared to females [14, 10]. Uric acid, on the other hand, constitutes a major water-soluble antioxidant in fish blood [30]. In this study, uric acid level was significantly higher in females compared to males. This study appears to be the first one that evaluates uric acid in male and female catfish, and the differences in uric acid levels between males and females could only be speculatively attributed to differences in the rate of excretion, mainly via the kidney, as well as from overproduction of uric acid owing to excess purine precursors synthesis, turnover of cells [31].

Blood electrolytes such as Sodium (Na^+), Potassium (K^+), Chloride (Cl^-), and Phosphorous (*P*) are common parameters employed in the determination of physiological states, toxicity, and health status of

fishes, and their levels reflect operations of diverse homeostatic mechanisms in fish [14]. In this study, Sodium, Chloride, Bicarbonate, and Potassium levels were higher in males than females, even though the differences were not statistically significant. In a similar study, Kulkarni [14] found that male Notopterus notopterus had lower levels of these electrolytes compared to males. The differences in the electrolyte levels were attributed to differences in sensitivity to environmental changes and strength [32, 14].

Conclusion

This study revealed for the first time the effects of sexual dimorphism on the serum chemistry and electrolyte profile of male and female H. longifilis. While there is no significant difference in the majority of analytes, there were few significant differences between males and females. Based on the findings of this study, it is recommended that sex be considered in both natural and experimental investigations of serum chemical analytes and electrolytes in catfish.

Materials and Methods

Experimental catfish

The 40 apparently healthy adult male and female catfish (20 males and 20 females) weighing 1 kg to 1.3 kilograms used for the study were procured from a reputable catfish farm within the Jos metropolis with GPS Coordinates 9.851095 (N905113.9416411) and 8.923327 (E8055125.2188411) at altitude 1275 meters above sea level. The catfish had no physical deformity and were acclimatized for one week in a section of the farm in a 2000-liter plastic water tank before the onset of the study. The fish were exposed to natural light day and night without any artificial light. The fish were fed commercial pelleted diets (Coppens) daily in the morning and evening at 10% of their body weight. The water in the tanks was changed every three days through partial draining to ensure that clean water was maintained during the acclimatization. For sampling, a handheld net was used to catch the fish from the tank without completely draining the water from the tank, and a soft, clean towel was used to hold the catfish in place during blood collection.

Experimental design

The 40 catfish were male and female. Following acclimatization, blood samples were collected from 20 male and 20 female catfish. The water used before and during the study was borehole water used in keeping the fish on the farm. The study was conducted in June 2022, and the water temperature during the study period ranged from 20°C to 24°C, pH ranged from 7.0 to 7.1, while the average environmental temperature was 26°C during the day and 18°C at night.

Separation of serum from the blood and analysis

Blood samples collected from the caudal vein into non-heparinized tubes were immediately transported to the Microbiology and pathology laboratory of the Faculty of Veterinary Medicine, University of Jos, Plateau State, where they were centrifuged and the sera were harvested after centrifugation for serum chemistry analysis using an automated serum chemistry analyzer, Cobas C111 (Roche Diagnostics GmbH, Indianapolis, IN, USA).

Statistical analysis

Data analysis was done using JMP statistical software, version 10. All data were found to have a normal distribution. Since two groups were compared, the student t-test was used to identify statistically significant differences between males and females. Differences between sexes were considered statistically significant at $p < 0.05$. Results are expressed as the mean ± standard error (SE).

Authors' Contributions

P.N.T., and G.B. conceived and planned the experiments. P.N.T., G.B., and M.M.S. carried out the experiments. P.N.T., G.B., and M.M.S. contributed to sample preparation. P.N.T., G.B. and M.M.S. contributed to the interpretation of the results. P.N.T. 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 have nothing to disclose.

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RESEARCH ARTICLE

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Prevalence of *Chlamydia abortus* Infection in Aborted Sheep and Goats in Kerman Province, Southeast of Iran

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ABSTRACT

In recent years, *C. abortus*, the etiological agent of ovine enzootic abortion, has been associated with many cases of lamb loss in sheep and goat farms in Iran. However, there is a lack of epidemiological data regarding Chlamydia-related abortion in this region. Accordingly, we aimed to investigate the prevalence of *C. abortus* and the associated risk factors in the small ruminants of Kerman Province, southeast Iran. For this purpose, we collected 134 vaginal swab samples from 70 sheep and 64 goats that had experienced abortion. Following DNA extraction from samples, we amplified the POMP90-3 gene of *C. abortus* using PCR to confirm *C. abortus* presence, and then one positive sample was selected for sequencing. The results indicated an overall C. abortus prevalence rate of 21.6%, with 20.3% prevalence in goats and 22.8% in sheep. We observed a higher incidence rate in animals with a higher number of parturition; however, no significant corelation was observed between the prevalence rate of *C. abortus* and species. In addition, sampling location was considered a risk factor associated with *C. abortus* infection. This study highlighted *C. abortus* as a threat to small ruminants' reproduction in Kerman Province, which deserves constant monitoring and multi-faceted preventive strategies.

Keywords

Chlamydia abortus, Kerman province, Ovine enzootic abortion, PCR

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Abbreviations

C. abortus: Chlamydia abortus
MOMP: Major outer membrane proteins
POMP: Polymorphic outer membrane proteins
PBS: Phosphate-buffered saline
PCR: Polymerase chain reaction
OEA: Ovine enzootic abortion

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Introduction

C. abortus, a Gram-negative bacterium belonging to the family Chlamydiaceae, is an obligate intracellular pathogen responsible for OEA or EAE. The disease burdens considerable economic loss in small ruminant farms if it affects enormous cases called abortion storms [1-4]. *C. abortus* transmits through any environmental exposure to the bacteria released by infected animals, abortion materials, or post-partum secretions, which poses health concerns for pregnant women and wild animals [5, 6]. Spillover of *C. abortus* through domestic and wild animal reservoirs has made controlling the disease difficult [5].

In the initial stage of *C. abortus* infection, bacteria colonize in the lymphatic tissues and then disseminate to other organs, resulting in several implications, such as pregnancy loss (abortion) and birth to stillborn if the infection occurred in the late stage of pregnancy (5-6 months) [3, 7-9]. Otherwise, bacteria enter the latency phase and may cause abortion in the second year of pregnancy [10]. Various approaches are available for confirming *C. abortus* in diagnostic laboratories. Methods for the direct identification of the agent, such as *C. abortus* isolation from clinical samples, staining the smears of fecal samples or vaginal swabs, and immunological staining of the organism, are either outdated or non-convenient [11-15]. Serological tests, including CFT and ELISA, are used for the indirect diagnosis of C. abortus [16]. These techniques identify the presence of chlamydial antibodies in the sera of infected animals. However, they have been replaced with molecular methods to improve the detection of C. abortus. Molecular identification methods, such as PCR, real-time PCR, and DNA microarray are highly sensitive approaches due to targeting different biomarker sequences, namely conserved regions, MOMP, POMP genes, or the intergenic space between the 16S and 23S rRNA genes [17-20].

Although *C. abortus* distributes worldwide, the reported distribution of C. abortus is far from the true infection prevalence [5] because of the variability in the sensitivity and specificity of the diagnostic tests and a lack of *C. abortus* epidemiological information, especially in developing countries in Asia and Africa [21]. OEA is endemic in Iran, and several studies previously reported the incidence of the disease in sheep and goats in some areas of this country [22-25]. In the present study, we attempted to investigate the prevalence rate and associated predisposing factors of *C. abortus* infection in aborted sheep and goats of Kerman Province in Iran to provide valuable insight into bacteria spillover in this region.

Abbreviations-Cont'd

EAE: Enzootic abortion of ewes
CFT: Complement fixation test
ELISA: Enzyme-linked immunosorbent assay

Result

Identification of C. abortus

Among 134 vaginal swabs collected from sheep and goats, 16 sheep (22.8%) and 13 goats (20.3%) were confirmed to be positive for C. abortus based on the amplification of the POMP 90-3 gene (220 bp) in PCR (Figure 1). The PCR results were validated by sequencing and blasting one PCR product, which showed the highest similarity with the POMP 90-3 gene of C. abortus that was previously registered on NCBI under the accession number ACD10929.1.

Prevalence of C. abortus infection

The prevalence rate of chlamydiosis based on different variables, such as animal species, age, number of parturition, and the location was statistically analyzed in the aborted flocks of sheep and goats using the Chi-squared test (Table 1). According to the results, the prevalence of *C. abortus* varied in the different regions ranging from 0% in Bam city to 28.3% in Baft city. Our findings revealed a significant correlation between the geographical area and the level of *C. abortus* in flocks ($p = 0.03$). There was no significant relationship between C. abortus infection and animal species (sheep and goats) ($p = 0.7$), or the age of infected animals ($p = 0.2$). However, the number of abortions in infected animals had a significant correlation with parity ($p = 0.001$).

Figure 1.
The agarose gel electrophoresis of the POMP 90-3 gene of *C. abortus* isolates.
M: 100 bp ladder; N: negative control (distilled water); P: positive control (*C. abortus*); lanes 1-17: test samples. The observation of a 220 bp band in a sample confirmed *C. abortus* presence.

Discussion

OEA is an infectious disease with clinical demonstrations in small ruminants, such as sheep and goats [11]. Due to massive economic loss, chlamydial abortion is a global concern in agricultural industries in Europe, North America, Africa, and Iran [21]. There are various laboratory diagnostic techniques for surveying the epidemiology of the disease, such as serological tests and basic detection methods, which provide less sensitivity and specificity for the confirmation of microorganisms. However, molecular methods based on outlining specific genes can reliably identify and differentiate the chlamydial species [17].

In the present study, we identified a high incidence rate of *C. abortus* infection in the Kerman Province of Iran, with ranges of 20.3% and 22.8% in goats and sheep, respectively. The results indicated that various factors, such as geographical location and the number of parturitions, could influence *C. abortus* infection. This observation also highlighted the need for constant genetic and antigenic evaluation of abortion isolates to establish national strategies for preventing the transmission of *C. abortus* in the future.

The prevalence rate of *C. abortus* infection in small ruminants depends on many factors, including the geographical location, size and type of samples taken, animal breed, grazing and management strategies, nutritional deficiency, uncontrolled restriction of a diseased animal movement from infected areas, choice of diagnostic antigen, and studding method [18]. Moreover, aging, species, gender, number of parturition, and geographical region are reported as effective factors in the prevalence of *C. abortus* [25]. Most investigations on the prevalence of OEA in sheep and goats reported an average rate of 20%-37% in Iran [22-25]. In this regard, a survey showed a twice higher prevalence in Chaharmahal and Bakhtiari province. However, some other studies reported a low prevalence of 9% in the south to 11% in the northeast of Iran [26-28]. In neighboring countries, such as Iraq, Arif et al. recorded chlamydiosis in only one of the 30 samples from the aborted ewes (3.3%) in Sulaimani province, which is far from the rate commonly reported in Iran [18]. In the current study, we observed an overall *C. abortus* prevalence of 21.6% among the small ruminants of Kerman province, which was in agreement with most available data in Iran. We also detected diverse incidence rates in different cities, which is consistence with the sero-prevalence of C. abortus in the countries of origin Jordan [29] and China [30]. In contrast with our study, the incidence rate of *C. abortus* infection showed no difference among populations located in different epidemiologic areas of Khorasan Razavi province, northeast of Iran [28]. Another research in the southwest of Iran also showed that the geographical origin of sheep had no significant effect on the incidence of *C. abortus* [31].

Our findings showed that chlamydial infection incidence was higher in ewes with a higher number of parturition. Other studies also reported similar results in Iran and Jordan [25, 29]. The establishment of the latent form of *C. abortus* pathogenesis in non-pregnant infected ewes and the bacteria reactivation and proliferation in the subsequent pregnancy might be the reason for the higher prevalence of infection in ewes with a higher parity [9].

According to Table 1, the age of animals is not a predisposing factor for *C. abortus* prevalence. In agreement with

Table 1.
Prevalence rate of C. abortus in sheep and goats from different regions of Kerman Province, Iran

Variables	No.	Number of positive samples	Prevalence (%)	*p-value*
Animal species:				
Sheep	70	16	22.8	
Goat	64	13	20.3	0.7[b]
Total	134	29	21.6	
Number of parturition:				
< 2	59	4	6.7	0.001[a]
2 – 4	40	10	25	
> 4	35	15	42	
Age (yr):				
< 2	45	12	26.6	
2 – 4	50	7	14	0.2[b]
> 4	39	10	25.6	
City:				
Baaft	60	17	28.3	
Bam	10	0	0	
Bardsir	25	4	16	0.03[a]
Kerman	14	2	14.2	
Shahr-e Babak	25	6	24	

a: significant difference ($P < 0.05$)
b: insignificant ($P > 0.05$)

this result, Iraninezhad et al. [28] and Cubero et al. [32] recorded no significant correlation between age and the epidemiology of chlamydial infection. Contrary to our study, a positive relationship between the age of aborted animals and the chance of positivity for *C. abortus* was mentioned in other reports [25, 30].

According to our results, although the chlamydial infection rate was higher in sheep than in goats, this difference was not significant (Table 1). Similarly, previous studies showed that species was not a risk factor for the occurrence of chlamydial infection [23, 25, 28, 30, 33]. In this regard, a difference was reported by other researchers in the infection incidence between sheep and goats [34-36]. For example, a higher rate of chlamydial infection was observed in sheep compared to goats in Taiwan [37].

Conclusion

This study was the first report on the prevalence of C. abortus infection among goats and sheep in Kerman province of Iran. According to PCR results, C. abortus was responsible for 22.8% and 20.3% of abortion incidence in sheep and goats, respectively. This finding indicates the circulation of *C. abortus* among small ruminants in Kerman province, which poses serious public health concerns.

Materials and Methods

Sample collection

During the lambing season of 2022, 134 vaginal swab samples were collected from 70 sheep and 64 goats with a history of abortion in different cities of Kerman province in the southeast of Iran. The samples were suspended in 500 µl of sterile PBS and then transferred on ice to the Microbiology Laboratory at the Faculty of Veterinary Medicine of the Shahid Bahonar University of Kerman. The samples were stored at -20°C for DNA extraction.

DNA extraction

The DNA was extracted from vaginal samples using DNA extraction commercial kit (Cinaclon, Iran) according to the manufacturer's instructions. The extracted DNA was quantified by a NanoDrop spectrophotometer (Epoch, BioTek Instruments Inc., USA) at the wavelength of 260 nm, and stored at -20°C for further analysis.

PCR verification

To detect C. abortus, PCR was performed on the extracted DNA to amplify the POMP 90-3 gene with specific primers (F:5'–TTTTCAGGATCCTATTGTCCTCCAGGCA–3' and R:5'–GTGAATTCATCAGCATAAATAGCCCCG–3') [14]. The PCR reaction mix was prepared at a final volume of 20 µl, including 10 µl master mix (Amplicon, Denmark), 4 µl template DNA, 0.5 µl of each forward and reverse primer, and 5 µl distilled water. The amplification was initiated with 3 min of denaturation at 95°C, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 30 seconds, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. the PCR amplicons were visualized by agarose gel electrophoresis 1.5% and exposed to a UV light to detect the POMP 90-3 gene (220 bp).

POMP 90-3 gene sequencing

In the next step, the PCR product of one Chlamydia-positive sample was subjected to sequencing (Macrogen Inc., South Korea) to confirm the amplified POMP 90-3 gene. After receiving DNA fragments of the POMP 90-3 gene, they were trimmed and then assembled using DNAstar software. The final consensus of the received sequence was compared to any relevant sequence in the NCBI database using BLAST.

Statistical analysis

The sample size was calculated using the online software https://www.calculator.net/sample-size-calculator.html, with a confidence level of 95% and desired absolute precision of 10%. The SPSS for Windows (version 25.0; IBM Corp., Armonk, USA) was applied to perform statistical analysis. The rate of abortion between the investigated groups was explained as percentage of all the sampled animals. The effect of independent risk factors, such as sampling location, number of parturition, animal species, and age on the prevalence of *C. abortus* infection was analyzed by Chi-squared test. The differences in prevalence were considered significant at $p < 0.05$.

Authors' Contributions

S.A. collected samples, carried out the analysis of samples, data analysis, and wrote the manuscript. M.G. designed the study, supervised the project, revised the data analysis, and critically revised all parts of the manuscript. E.M. supervised the laboratory works. N.E. formal analysis, writing—review and editing. M.A.S. formal analysis, writing—review and editing.

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Competing Interests

The authors declare that there is no conflict of interest.

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RESEARCH ARTICLE

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Production performance of kampung hens fed rations containing black soldier fly larvae powder

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ABSTRACT

This research aimed to find a suitable formulation for rations containing Black soldier fly larvae powder to support the optimal kampung hen production performance. Four to five-month-old Kampung hens were given feed with different amounts of black soldier fly larvae powder (n = 4 hens/treatment) to adjust protein and energy levels: R0 (commercial feed only, 17.53% protein, 3067 kcal/kg), R1 (14% protein, 2600 kcal/kg energy), R2 (14% protein, 2800 kcal/kg energy), R3 (16% protein, 2600 kcal/kg energy), R4 (16% protein, 2800 kcal/kg energy), R5 (18% protein, 2600 kcal/kg energy), R6 (18% protein, 2800 kcal/kg energy), R7 (20% protein, 2600 kcal/kg energy), R8 (20% protein, 2800 kcal/kg energy), R9 (22% protein, 2600 kcal/kg energy), and R10 (22% protein, 2800 kcal/kg energy). We measured several performance parameters, including body weight, feed consumption, specific growth rate, feed conversion ratio, visceral index, intraperitoneal fat index, and tissue protein content. Results show that treatment R6 produced significantly better overall performance ($p < 0.05$) than all other treatments except R5. Feed containing black soldier fly larvae powder with 18% protein content and 2800 kcal/kg energy is an inexpensive and readily available way to support the maximum growth of Kampung hens.

Keywords

Feed quality; Metabolic energy; Production performance; Livestock; Kampung hen; Poultry feed

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Abbreviations

BSF: black soldier fly
SGR: Specific Growth Rate
FCR: Feed Conversion Rate
VSI: Visceral index
IFI: Intraperitoneal Fat Index

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Introduction

Good quality feed is a pivotal factor determining the success of livestock production and constitutes a significant portion of the costs in the livestock industry. In poultry farming, feed costs can account for 50-70% of the total operational cost [1]. Therefore, there is a need for cost-cutting methods that do not compromise on quality. Using high-quality feed has been demonstrated to enhance the productivity of Kampung chickens [2,3]. Good feed quality is determined by its ability to supply the animal's nutritional requirements (i.e., protein, carbohydrates, fat, vitamins, and minerals). The quality of the feed is also determined by its composition and balance of nutrients [4]. Moreover, good quality feed must be available year-round to maintain optimal performance and production. Sustainable feed provision is reliant on the availability of raw materials.. Specifically, the raw materials of feed must be easy to obtain, relatively cheap, not compete with human needs, and have a high nutrient content. Feed quality is directly related to feed efficiency. Optimization of feed efficiency is dependent on the formulation of balanced rations, especially concerning protein and energy. The right balance between protein and energy in a ratio increases the efficiency of feed use by the animal, thereby reducing overall production costs [5].

Alternative raw materials for poultry feed that are cheap and readily available include components of agricultural or insect waste. According to van Huis [6], using insect proteins in feed is cost-effective and eco-friendly. Insects can quickly and easily be mass-produced and have a high feed conversion efficiency. Cultivation of insects could reduce the amount of organic waste that potentially pollutes the environment [7]. Furthermore, Veldkamp et al. [8] also reported that using insects as a protein source for feed was beneficial because it does not compete with human needs. One insect that has been widely studied as a protein source in feed is the black soldier fly (BSF) due to its high protein (40–50%) and fat (29–32%) content [9]. BSF larvae powder is a suitable alternative feed additive for broiler chickens [10–12], Jian carp fish [13], and quail [14–16].

Kampung chicken has low productivity but has high economic value, especially eggs and meat. The demand for kampung chicken meat and eggs in Indonesia has increased yearly. Fitri [17] reports that the consumption of Kampung chicken meat in 2015 was only 314 thousand tons (16%) of the total meat production of 3.06 million tons, and in 2016 increased to 26%. It is further stated that this demand will continue to increase in line with population growth and awareness of the importance of organic food products. Efforts are needed to increase the productivity of Kampung chickens to meet this increasing demand. One way to increase the productivity of Kampung chickens is to provide quality feed according to the age of the chickens. Charlton et al. [18] analyzed the security of some insects as a source of protein in feed livestock, such as house flies (Musca domestica), Bluebottle flies (Calliphora vomitoria), blowflies (Chrysomyaspp), and BSF. Therefore, the present study evaluated the effects of feed containing BSF larvae powder as a protein source on the production performance of Kampung hen.

Result

Effect of treatment on body weight

Table 3 and Figure 1a illustrate that the greatest change in body weight was obtained with R6, whereas treatment R0 elicited the smallest change. The change in body weight obtained with R6 was significantly different from all other treatments except R5.

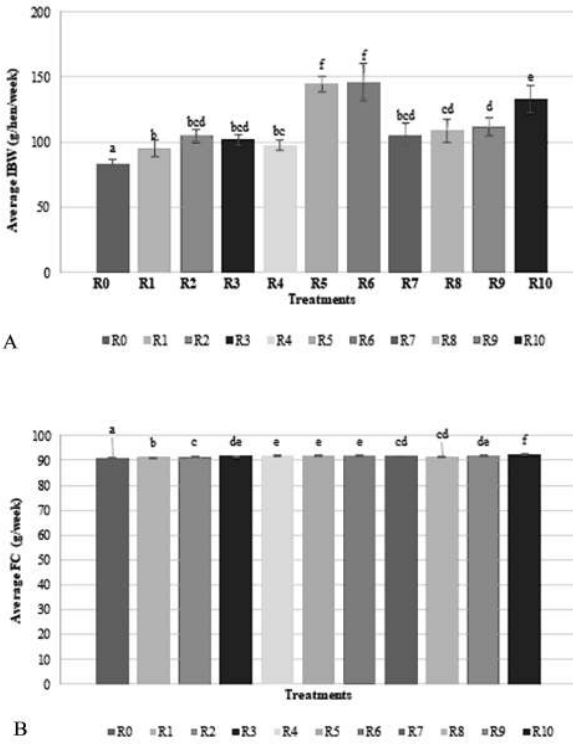

Figure 1.
A. Average increase in body weight (IBW; g/hen/week) during an eight-week observation period. **B.** Average feed consumption (FC; g/week) during an eight-week observation period. Different letters (a,b,c,d,e,f) indicate significant differences ($p < 0.05$).

Table 3.
Amino acid analysis of the experimental feed

Feed	R0 (%)	R1 (%)	R2 (%)	R3 (%)	R4 (%)	R5 (%)	R6 (%)	R7 (%)	R8 (%)	R9 (%)	R10 (%)
Aspartic acid	1.00	1.01	1.03	1.15	1.26	1.29	1.38	1.49	1.58	1.68	1.88
Glutamic acid	1.68	1.74	2.54	2.61	2.76	2.89	3.33	3.46	3.37	3.43	3.78
Serine	0.43	0.48	0.48	0.57	0.64	0.72	0.87	0.89	0.89	0.93	1.00
Glycine	0.77	0.81	0.83	0.88	0.94	1.13	1.24	1.21	1.26	1.29	1.32
Histidine	0.54	0.60	0.63	0.70	0.78	0.81	0.79	0.77	0.82	0.90	0.91
Arginine	0.67	0.66	0.70	0.69	0.72	0.79	0.75	0.70	0.78	0.82	0.88
Threonine	0.53	0.59	0.68	0.71	0.66	0.75	0.82	0.81	0.83	0.85	0.92
Alanine	0.41	0.48	0.52	0.57	0.54	0.69	0.76	0.75	0.78	0.80	0.83
Proline	0.84	0.90	0.92	0.95	1.12	1.20	1.15	1.18	1.20	1.21	1.37
Tyrosine	0.74	0.79	0.78	0.83	0.90	0.88	0.91	0.93	1.00	1.05	1.15
Valine	0.66	0.70	0.90	0.86	0.80	0.77	0.73	0.69	0.80	0.82	0.87
Methionine	0.50	0.47	0.52	0.60	0.65	0.70	0.71	0.67	0.72	0.76	0.81
Cystein	0.31	0.37	0.42	0.52	0.50	0.54	0.59	0.60	0.63	0.68	0.71
Isoleucine	0.67	0.70	0.73	0.80	0.82	0.76	0.79	0.80	0.80	0.83	0.88
Leucine	1.01	1.17	1.22	1.30	1.30	1.29	1.30	1.36	1.32	1.38	1.51
Phenylalanine	0.52	0.57	0.56	0.67	0.70	0.67	0.69	0.72	0.75	0.77	0.88
Lysine	0.75	0.82	0.79	0.84	1.00	1.04	1.13	1.18	1.25	1.29	1.34

Analysis was performed by the Feed Science and Technology Laboratory, IPB-Faculty of Animal Husbandry, Department of Nutrition Science and Feed Technology, Division of Feed Technology and Industry.

Effect of treatment on feed consumption

The results showed that the different feed treatments had a significant effect on the consumption of feed ($p < 0.05$). In particular, the consumption of feed R6 was significantly higher than all others, while the consumption of R0 was significantly lower. Importantly, heightened consumption of R10 was not followed by a high growth rate (Table 3 and Figure 1b). Results of Amino acid analysis revealed that all the experimental feeds contained essential and nonessential amino acids, albeit at different concentrations (Table 4).

Effect of treatment on SGR (Specific Growth Rate)

As with changes in body weight, the SGR of chickens treated with feed R6 was significantly higher ($p < 0.05$) than all other feeds except R5 (Table 3 and Figure 2a).

Effect of treatment on FCR (Feed Conversion Rate)

The results showed the feed treatments had significant effects on the FCR ($p < 0.05$; Table 3 and Figure 2b). Body weight Changes and SGR values are

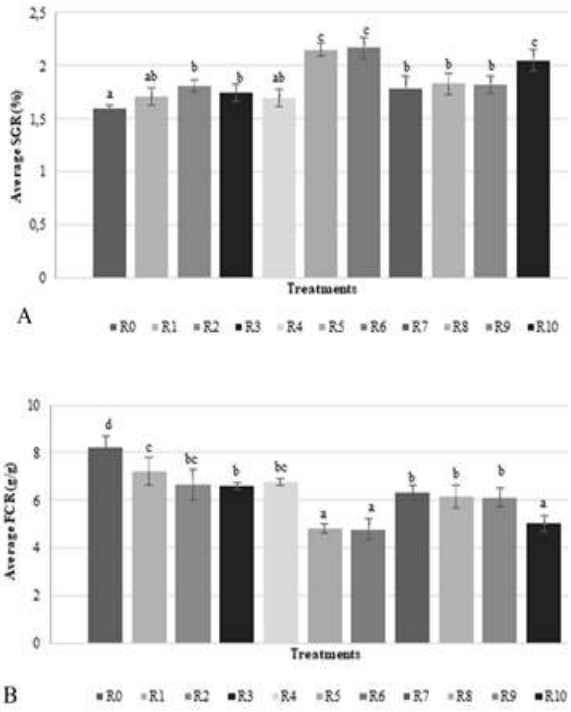

Figure 2.
A. Average SGR (%) during an eight-week observation period. **B.** Average feed conversion ratio (FCR; g/g)) during an eight-week observation period. Different letters (a, b, c, d) indicate significant differences ($p < 0.05$).

determined by the FCR, not feed consumption, as shown by the current results (Table 3).

Effect of treatments on VSI (Visceral index)

The VSI obtained with treatment R6 was significantly higher ($p < 0.05$) than that with all others except R5 (Table 5 and Figure 3). This high VSI was due to the high body weight achieved by the end of the experimental period. While feed R0 had the lowest VSI, it was not significantly different ($p > 0.05$) with R2, R3, or R7–R10.

Effect of treatments on IFI (Intraperitoneal Fat Index)

The IFI for treatment R10 was significantly higher than all other feeds ($p < 0.05$; Table 5 and Figure 4). The high IFI R10 indicated that a feed with 22% protein and 2800 kcal/kg energy had excess nutrition for chickens during the growth phase, which was converted into intraperitoneal fat. R10 also had the highest feed consumption (Table 1), further contributing to the high IFI. This result was supported by the lower tissue protein content obtained with treatment R10 (Table 5).

Table 4.
Increase in body weight (IBW), feed consumption (FC), specific growth rate (SGR), and feed conversion rate (FCR) during an eight-week-observation period

Treatments	IBW (g/ week)	FC (g/week)	SGR (%)	FCR (g/g)
R0	83.87 ± 3.27^{a}	90.96 ± 0.31^{a}	1.59 ± 0.04^{a}	8.23 ± 0.47^{d}
R1	95.44 ± 5.30^{b}	91.32 ± 0.06^{b}	1.71 ± 0.08^{ab}	7.22 ± 0.57^{c}
R2	105.09 ± 4.93^{bcd}	91.62 ± 0.09^{c}	1.81 ± 0.06^{b}	6.66 ± 0.63^{bc}
R3	102.13 ± 3.94^{bcd}	91.85 ± 0.05^{de}	1.74 ± 0.08^{b}	6.58 ± 0.14b
R4	97.81 ± 3.82^{bc}	92.07 ± 0.12^{e}	1.70 ± 0.08^{ab}	6.75 ± 0.13^{bc}
R5	144.25 ± 6.03^{f}	91.89 ± 0.14^{de}	2.15 ± 0.06^{c}	4.83 ± 0.19^{a}
R6	145.81 ± 14.29^{f}	91.90 ± 0.09^{de}	2.17 ± 0.10^{c}	4.77 ± 0.46^{a}
R7	105.75 ± 8.71^{bcd}	91.75 ± 0.08^{cd}	1.78 ± 0.12^{b}	6.32 ± 0.34^{bc}
R8	108.81 ± 8.64^{cd}	91.69 ± 0.20^{cd}	1.84 ± 0.10^{b}	6.14 ± 0.46^{bc}
R9	111.84 ± 7.07^{d}	91.92 ± 0.05^{de}	1.83 ± 0.08^{b}	6.10 ± 0.39^{b}
R10	133.31 ± 10.15^{e}	92.42 ± 0.05^{f}	2.05 ± 0.10^{c}	5.02 ± 0.33^{a}

Data in a column with different superscripts (a, b, c, d, e, f) differ significantly ($p < 0.05$).

Effect of treatments on tissue protein content

The tissue protein content obtained with feed R5 was significantly higher ($p < 0.05$) than all others except R6, while R1 produced the lowest (Table 5 and Figure 5).

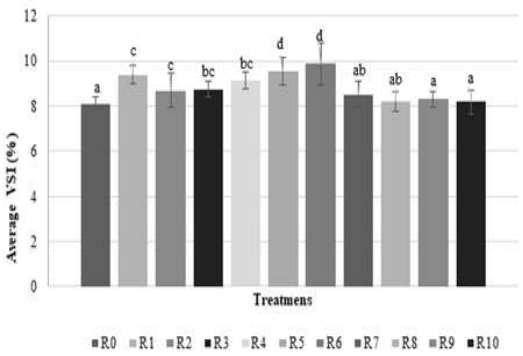

Figure 3.
Average visceral index (VSI; %) during an eight-week observation period. Different letters (a, b, c, d) indicate significant differences ($p < 0.05$).

Table 5.
Visceral Index (VSI), Intraperitoneal Fat Index (IFI), and tissue protein content of each treatment

Treatments	VSI (%)	IFI (%)	Tissue Protein Content (%)
R0	8.07 ± 0.33^{a}	1.11 ± 0.05^{a}	21.48 ± 0.77^{e}
R1	9.39 ± 0.42^{cde}	1.77 ± 0.08^{c}	17.83 ± 0.16^{a}
R2	8.69 ± 0.78^{abcd}	1.43 ± 0.04^{b}	19.49 ± 0.15^{bc}
R3	8.75 ± 0.35^{abcd}	1.66 ± 0.03^{c}	21.21 ± 0.86^{e}
R4	9.15 ± 0.37^{bcd}	1.73 ± 0.02^{c}	18.78 ± 0.16^{ab}
R5	9.55 ± 0.639^{de}	1.34 ± 0.03^{b}	26.36 ± 0.21^{g}
R6	9.87 ± 0.94^{e}	1.39 ± 0.11^{b}	25.296 ± 0.50^{g}
R7	8.51 ± 0.59^{abc}	1.68 ± 0.04^{c}	20.66 ± 0.13^{de}
R8	8.21 ± 0.44^{a}	1.88 ± 0.10^{d}	24.39 ± 0.34^{f}
R9	8.30 ± 0.37^{ab}	1.91 ± 0.10^{d}	19.85 ± 0.42^{cd}
R10	8.18 ± 0.51^{a}	2.11 ± 0.09^{e}	19.79 ± 0.27^{bcd}

Different superscript (a, b, c, d, e, f, g) in the same column indicates data significantly different ($p < 0.05$).

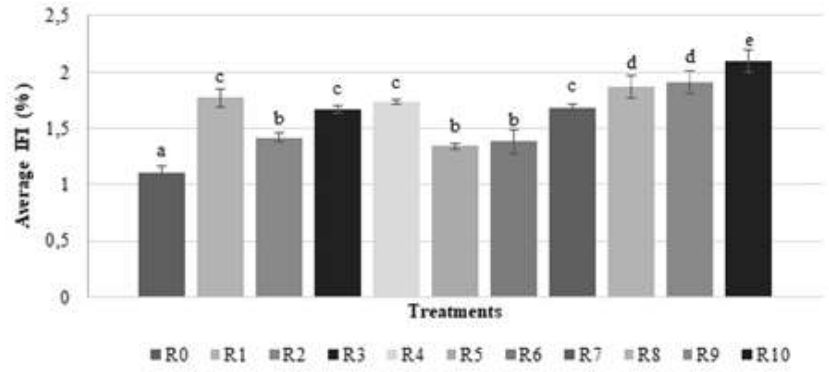

Figure 4.
Average intraperitoneal fat index (IFI, %) during an eight-week observation period. Different letters (a, b, c, d, e) indicate significant differences ($p < 0.05$).

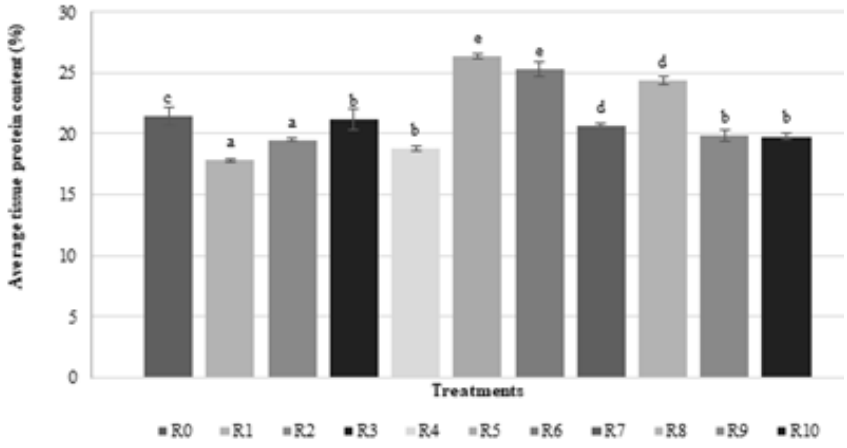

Figure 5.
Average tissue protein content (%) during an eight-week observation period. Different letters (a, b, c, d, e) indicate significant differences ($p < 0.05$).

Discussion

Effect of treatment on body weight

These results are similar to a study by Abun et al. [19], who found that Kampung chickens fed 2750 kcal/kg energy and 17% protein had the best production performance. Similarly, Sidadolog and Yuwanta [4] found the greatest change in body weight for Marawang chicks was with feed containing 18% crude protein and 2690 kcal/kg energy.

The high increase in body weight with R6 corresponded to a low feed conversion ratio and high SGR. The lack of significant difference in body weight change between R5 and R6 indicates that feeds containing the same protein level likely result in similar changes in body weight, assuming the energy content is also balanced. However, diets with higher protein and metabolic energy levels did not necessarily equate to heavier body weight. This result indicates that optimum growth is obtained only with the right balance between protein and energy corresponding to the physiological condition of the animal, meaning the animal was able to maximize the conversion of consumed feed into biomass [20]. This is in agreement with Tortora and Grabowski in Abun et al. [19], who stated that the balance between the ratio of protein and energy and the presence of other nutrients in the feed plays an important role in maximizing body weight gains.

Protein quality is determined by the amino acid composition, especially the essential amino acids. Hence, the more complete and appropriate the amount of essential amino acids, the better the protein quality of the feed. In particular, methionine and lysine are the amino acids most needed for chicken growth; the reported maximal requirements of methionine and lysine in poultry diets are 0.38-0.42% and 0.8-1.0%, respectively [5]. Though experimental feed R6 contained 0.71% methionine and 1.13% lysine, other feeds contained higher levels. This suggests that higher concentrations of amino acids in the feed do not guarantee greater changes in body weight.

Effect of treatment on feed consumption

Increased consumption of R10 did not result in a high growth rate. This was likely due to its high FCR

compared to R6. Such a high FCR indicates the limited ability to convert consumed feed into biomass and suggests protein and energy levels may be greater than the needs of the animal, with the excess being excreted as waste [5]. On the other hand, while consumption of feeds R3, R5, R6, and R9 was not significantly different, these feeds did result in different body weight changes. This indicates that body weight change did not directly correlate with feed consumption. Sidadolog and Yuwanta [4] stated that body weight correlates with feed conversion rate, not feed consumption.

Effect of treatment on SGR (Specific Growth Rate)

SGR values were inversely correlated with the feed conversion ratio, where the lower the feed conversion ratio, the higher the SGR. Importantly, higher protein and energy levels did not guarantee a high SGR, indicating that optimum growth was reached only when rations contained protein and energy levels appropriate for the physiological conditions of the animal. This result was in agreement with the results of Abun et al. [19], who stated that an appropriate balance between protein and energy in the feed has a positive effect on growth.

Effect of treatment on FCR (Feed Conversion Rate)

The FCR represents the ability of the animal to convert consumed feed into biomass; a low FCR indicates a higher ability to convert the consumed ration into biomass and vice versa. Feed R6 had a significantly lower FCR than all others except R5, and the highest was obtained with R0. An FCR of 4.77 (R6) indicates that chickens consuming feed with 18% protein and 2800 kcal/kg energy needed to consume 4.77 kg feed to increase their body weight by 1 kg. The absence of significant difference between treatments R5 and R6 implies both produced the same change in body weight. This result is similar to that of Iskandar et al. who obtained an FCR of 4.79 for Kampung chickens fed a ratio of 17% protein [21]. On the other hand, Mahardika et al. [5] obtained an FCR of 9.39 by feeding 10- to 20-week-old Kampung chickens containing 18% protein and 2900 kcal/kg metabolic energy , which was also in accordance with a report by Sidadolog and Yuwanta [4].

Effect of treatments on IFI (Intraperitoneal Fat Index)

These present results were higher than the resultsobtained by Iskandar et al. [21], who reported an abdominal fat index of 0.82% for Kampung hen. This difference could be due to genetic differences in chicken strains that are related to protein and metabolic energy level differences.

Effect of treatments on tissue protein content

The high tissue protein content with feeds R5 and R6 corresponded to their low feed conversion ratio and high increase in body weight. This indicates that a feed with 18% protein and 2800 kcal/kg contained the required nutrition, especially protein, that was optimal for Kampung hens during their growth phase. This result was similar to that reported by Abun et al. [19], who stated that high protein quality affects muscle protein.

Conclusions

The results of the present study showed that feed containing BSF larvae powder at 18% protein and 2800 kcal/kg energy (R6) contains balanced, adequate nutrition to support the optimal growth of Kampung hens. Based on the findings of this research, using feed with 18% crude protein and 2800 kcal/kg of metabolic energy results in the optimal growth of kampung hens.

Materials and Methods

Time and place of research

This research was conducted in Kupang, East Nusa Tenggara, from July to November 2018.

Animal treatment

A total of 44 Kampung hens (Gallus gallus domesticus) aged 4 to 5 months old were used. All Kampung hens were reared in-house and individually placed in 50 × 50 × 70 cm cages containing bowls for feed and water. The Kampung hens received feed containing different BSF larvae powder treatments (n = 4 hens/treatment). Hens initially received 70 g of feed, and subsequent amounts of feed added were adjusted based on the remaining amounts of feed. Water was given ad libitum.

Experimental feed

The raw materials present within the feed included BSF larvae powder, cornmeal, soybean meal, tapioca flour, fish flour, rice bran, and premix. Premix Composition (in 10 kg): Calcium 4.500 g, Sodium 800 g, Mangan 33 g, Phosphor 3.500 g, Magnesium 297 g, Ferrum 44 g, Zincum 33 g, Cholin 750 g, Cobalt 100 mg, Cuprum 5.500 mg, Iodine 550 mg, Vitamin B1 1.500 mg, Vitamin A 7.500.00 I.U dan Vitamin D3 1.500.000 I.U. Each ingredient was analyzed for its crude protein content and metabolizable energy (Bomb Calory Meter) [22,23], as a basis for formulating the feed. The dried BSF larvae, cornmeal, soybean meal, and fish purchased from the marketplace are blended into flour without reducing fat for BSF. BSF larvae and fish meal as a source of animal protein; soybean meal as a source of vegetable protein; Cornflour, rice bran, and tapioca as a source of carbohydrates, and Premix as a source of vitamins and minerals.

Experimental feed treatments included different amounts of BSF larvae powder (R1–R10) to adjust protein and energy levels as follows: R0 (commercial feed without BSF larvae powder, 17.53%

protein, 3067 kcal/kg energy), R1 (14% protein, 2600 kcal/kg energy), R2 (14% protein, 2800 kcal/kg energy), R3 (16% protein, 2600 kcal/kg energy), R4 (16% protein, 2800 kcal/kg energy), R5 (18% protein, 2600 kcal/kg energy), R6 (18% protein, 2800 kcal/kg energy), R7 (20% protein, 2600 kcal/kg energy), R8 (20% protein, 2800 kcal/kg energy), R9 (22% protein, 2600 kcal/kg energy), and R10 (22% protein, 2800 kcal/kg energy). Proximate and amino acid analyses were performed on the formulated feed with the desired protein and energy levels. Feed composition, proximate analysis, and amino acid analysis results are shown in Tables 1, 2, and 3, respectively. The duration of the experimental feeding period was a total of 8 weeks.

Table 1.
Composition of feed

Raw material (kg)	ME 2600 kcal/kg					ME 2800 kcal/kg				
	14%	16%	18%	20%	22%	14%	16%	18%	20%	22%
BSF powder	9	12	15	18	21	9	12	15	18	21
Corn flour	31.67	26.63	21.59	16.55	11.51	45.59	40.55	35.51	30.47	25.43
Soy flour	5	5	5	5	5	5	5	5	5	5
Tapioca flour	5	5	5	5	5	5	5	5	5	5
Premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Fish flour	0.34	2.79	5.23	7.67	10.12	2.17	4.62	7.06	9.50	11.95
Rice bran	48.49	48.09	47.68	47.28	46.87	32.74	32.34	31.93	31.53	31.12
Total	100	100	100	100	100	100	100	100	100	100
ME, kcal/kg	2600	2600	2600	2600	2600	2800	2800	2800	2800	2800
PC, %	14.0	16.0	18.0	20.0	22.0	14.0	16.0	18.0	20.0	22.0

ME = metabolizable energy; PC = crude protein

Table 2.
Proximate composition analysis of the experimental feed

Feed	% DM	% Ash	% OM	% CP	% CL	% CF
R0	89.94	4.07	85.87	16.762	6.44	5.06
R1	89.72	7.58	82.14	13.840	4.84	14.16
R2	88.98	6.09	82.89	14.127	4.91	12.59
R3	89.31	7.35	81.96	15.645	4.63	12.57
R4	88.78	6.58	82.20	15.825	5.03	12.27
R5	90.02	8.67	81.35	17.951	8.43	14.90
R6	88.78	6.44	82.34	17.742	7.44	10.60
R7	89.48	8.04	81.44	20.375	8.28	14.03
R8	89.14	7.59	81.55	19.861	8.17	11.40
R9	89.41	9.26	80.15	22.401	8.22	13.63
R10	89.01	7.90	81.10	22.670	8.87	10.59

Analysis was performed at the Nutrition and Livestock Feed Laboratory, Agriculture Polytechnic, Nusa Cendana University, Kupang. DM: dry matter, Ash, OM: organic matter, CP: Crude protein, CL: Crude Lipid, CF: Crude fiber.

Authors' Contributions

J.J.B., A.B., and A.R.conceived and planned the experiments. J.J.B., A.B., and A.R. carried out the experiments. J.J.B. and A.B. contributed to sample preparation. A.B., A.B. contributed to the interpretation of the results. J.J.B. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Acknowledgements

Not applicable

Competing Interests

The authors declare that there is no conflict of interest.

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CASE REPORT

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The First Report of a Horned Owl Infection with the Trematode *Plagiorchis noblei* in Shahrekord, Iran

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ABSTRACT

In 2022, the trematode Plagiorchis noblei was isolated from a Horned owl (Asio otus) in Shahrekord city. This trematode was isolated from the small intestine of the Horned owl. The trematode was fixed on a microscope slide and stained with Carmine acid. Then the sample was examined with a stereomicroscope and identified with the available diagnostic keys. Studies showed that this trematode belongs to the species *Plagiorchis noblei*. This parasite belongs to the *Plagiorchiidae* family, but so far this parasite has not been observed in owls in Shahrekord city. This parasite belongs to the Digenea order, which causes lung, digestive, liver and blood diseases in birds and other vertebrates.

Keywords

Asio otus, Plagiorchiidae, Plagiorchis noblei, Shahrekord, Trematoda

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Abbreviations

No abbreviations

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Introduction

A special trematode species was first isolated and identified in the intestine of a Horned owl (*Asio otus*) in Shahrekord, Iran. Studies suggested that the trematode was from the genus *Plagiorchis noblei* belonging to the Plagiorchiidae family. The present case is the first report of a Horned owl infection with trematode *P. noblei* in Shahrekord.

Raptorial birds represent the top predators in the food chain and contribute significantly to the biological balance of nature given their unparalleled role in a healthy ecosystem [1]. They prey on insects, small mammals, and other birds. Most owls are nocturnal and solitary animals. The Horned owl, with the scientific name *Asio otus*, is a native owl of Asia, North America, and Europe. Little information is available on the parasitic disease prevalence in the wild owl population. Although these birds are prone to parasitic and other infections, they generally show no clinical symptoms [2].

Case Presentation

An infected Horned owl was collected by the General Department of Environmental Protection and was transferred to the Veterinary clinic at Shahrekord University, and died a few days after treatment. The owl was then dissected to isolate the digestive system, which was sent over to the Parasitology Laboratory at the Faculty of Veterinary Medicine, Shahrekord University. The digestive system was cut open and its content was transferred into a petri dish. Distilled water was added to the petri dish, and the parasite was detected and isolated using a stereomicroscope. Internal parasites were then isolated and placed in a glass container containing 5% glycerol and 70% alcohol. The trematodes were stained using carmine acid and were identified using the available diagnostic key [3].

Discussion

The identified trematode belonged to the Plagiorchis order, Plagiorchiidae family, and Plagiorchis genus. The mature helminth was 1.70 mm in length and 0.55 mm in width. The oral sucker of the parasite was almost spherical with a dimension of 220 × 270 µm (Figure 1). The abdominal sucker was revealed to be 160 × 190 µm. The helminth had two spherical testes behind an ovary and belonged to the Digenea order known to cause various digestive, hepatic, pulmonary, and blood diseases in birds and other vertebrates. Plagiorchis is a genus from the Plagiorchiidae family and a parasite affecting most vertebrates, such as mammals and birds.

Owls are valuable birds of prey playing the role of natural pest controllers. Similar to other birds, owls are prone to various infectious and non-infectious diseases, including bacterial (dermatophilosis), viral (Herpesvirus), and parasitic (Trichomonas) infections [4]. Mortality has been reported in birds due to infection with the studied parasite. Infection with this parasite prevents weight gain and causes depression and, ultimately, death in birds [5].

Ethics and animal experimentation

All animal experiments were performed in strict accordance with the guidelines approved by the Animal Ethics Committee of the Shahrekord University of Shahrekord. Iran.

Figure 1.
Microscopic image of the *Plagiorchis noblei* in the intestine of *Asio otus*

Authors' Contributions

Nader Ahmadi Saleh Baberi: Supervision, Conceptualization, Visualization, resources, Writing- Reviewing and Editing Reyhaneh Ghasemi: Supervision, Methodology, investigation, Resources, Writing- Reviewing and Editing. Navid Emami: Validation Hajar Sohrabinia : Validation. Resources,

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Competing Interests

The authors declare that there is no conflict of interest.

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Abstract in Persian

بررسی انگل‌های مدفوعی حیوانات خانگی غیرمعمول ارجاعی به بیمارستان تخصصی دام‌های کوچک دانشکده دامپزشکی دانشگاه تهران، ایران

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چکیده

حیوانات خانگی غیرمعمول، از پستانداران کوچک گرفته تا خزندگان، بخش قابل توجهی از مراجعان دامپزشکی را تشکیل می‌دهند. عفونت‌های انگلی ممکن است برای حیوان و برای صاحب آن تهدیدی بالقوه محسوب گردند. این مطالعه با هدف بررسی انگل‌های گوارشی که در طب حیوانات غیرمعمول امکان مواجهه با آن‌ها وجود دارد، انجام گرفته‌است. به این منظور نمونه مدفوع حیوانات خانگی غیرمعمول جمع‌آوری و از نظر وجود اجرام انگلی به صورت ماکروسکوپی و میکروسکوپی با گسترش مرطوب، تغلیظ با کمک شناورسازی و رنگ‌آمیزی اختصاصی بررسی شدند. ۳۰۰ نمونه مدفوع شامل ۲۶۲ پستاندار کوچک، ۳۷ خزنده و ۱ پریمات مشتمل بر لاگومورفا (۱۸۹/۳۰۰؛ ٪۶۳)، جوندگان (۳۰۰/۶۸؛ ٪۲۲.۶۶)، خزندگان (۱۸۹/۳۷؛ ٪۱۲.۳۳)، جوجه‌تیغی (۳۰۰/۴؛ ٪۱.۳)، یک شوگرگلایدر و یک عدد ماروموست در این مطالعه مورد نمونه‌برداری قرارگرفتند. در مجموع ۳۹ نمونه (٪۱۳) با حداقل یک انگل گوارشی آلوده بودند. انگل‌های مشاهده‌شده در این مطالعه شامل اُاُسیست، تخم‌های استرونگلی، تخم‌های اکسیورید (*Passalurus ambiguous*) و تخم‌های سستود بودند. یک نمونه از یک خوکچه هندی نیز آلوده به *Cryptosporidium sp.* تشخیص داده‌شد. با توجه به تغییر مداوم گونه‌ها و نیز منابع ناشناخته‌ی تهیه و تأمین حیوانات خانگی، و همچنین با درنظر داشتن احتمال بیماریزایی برخی گونه‌های انگلی برای انسان، بررسی دوره‌ای تنوع گونه‌ای حیوانات خانگی غیرمعمول شایع و آلودگی انگلی آنها اجتناب ناپذیر است. انگل‌های روده‌ای تحت بالینی در حیوانات خانگی ممکن است در صورت همراهی با مدیریت نامناسب، سلامتی حیوان همراه را متأثر نمایند. معمولاً در حیواناتی که علائم بالینی ندارند نیازی به درمان ضد انگلی وجود ندارد، اما می‌توان آزمایش‌های دوره‌ای انگلی را برای ارائه خدمات دامپزشکی صحیح توصیه نمود.

واژگان کلیدی

حیوانات خانگی، انگل‌های کرمی، ایمریا، کریپتوسپوریدیوم، هایمنولپیس، حیوانات خانگی غیرمعمول

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Abstract in Persian

مداخله درمانی در لنفادنیت کازئوز با استفاده از تزریق داخل آبسه‌ای ازن یا پراکسید هیدروژن در نشخوارکنندگان کوچک

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چکیده

لنفادنیت کازئوز بیماری‌ای زئونوز با خسارت اقتصادی مهم در جهان است. فعلاً مداخلهٔ جراحی و تخلیه آبسه تنها روش درمانی برای این بیماری است. در این مطالعه کارایی درمانی تزریق داخل آبسه‌ای " ازن در روغن زیتون" و "پراکسید هیدروژن در گلیسرین" در لنفادنیت کازئوز بررسی شد. ۱۸۰ راس گوسفند و بز مبتلا به لنفادنیت کازئوز به پنج گروه تقسیم شدند: ۱) کنترل منفی ۲) کنترل مثبتِ گروه ۱ (تزریق روغن زیتون) ۳) کنترل مثبتِ گروه ۲ (تزریق گلیسیرین) ۴) تزریق ازن محلول در روغن زیتون ۵) تزریق پراکسیدهیدروژن محلول در گلیسیرین. پیش از تزریق، نمونهٔ محتویات از نزدیکی جدار آبسه برای کشت میکربی اخذ شد. حجم آبسه‌ها قبل از تزریق (زمان صفر)، و دو هفتهٔ بعد (زمان ۱)، و چهار هفتهٔ بعد (زمان ۲) اندازه‌گیری شد. نتایجِ اندازه‌گیری حجم آبسه در زمان صفر و زمان ۲ به شرح زیر است: کنترل منفی (۰/۵ ± ۲/۹ ؛ ۰/۵ ± ۳/۵)،کنترل مثبتِ گروه ۱ (۰/۷ ± ۳/۴؛ ۱ ± ۶/۶)، کنترل مثبت گروه ۲ (۰/۷ ± ۳/۱؛ ۰/۹ ± ۳/۳)، گروه ازن (۰/۴ ± ۳/۳؛ ۰/۴ ± ۰/۴)، گروه پراکسیدهیدروژن (۰/۴ ± ۴/۶؛ ۰/۴ ± ۱/۵). نتایج نشان داد که حجم آبسه‌ها به میزان معنی‌داری تنها در گروه‌های درمانی کاهش یافته است. کورینه‌باکتریوم سودوتوبرکولوزیس تنها در ۴۸/۳ درصد از نمونه‌های باکتریولوژیک جدا شد. نتایج نشان می‌دهد که "ازن در روغن زیتون" و "پراکسیدهیدروژن در گلیسیرین" در تحلیل و پسرفتِ آبسه‌های لنفادنیت کازئوز کارآیی داشته‌اند. از نتایج چنین برمی‌آید که ازن در میزان و سرعت کاهش و پسرفت آبسه‌ها کارآیی بالاتری داشته است.

واژگان کلیدی

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شیوع عفونت کلامیدیا آبورتوس در گوسفند و بز سقط شده در استان کرمان، جنوب شرق ایران

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چکیده

در سال های اخیر، کلامیدیا آبورتوس (*C. abortus*)، عامل سقط جنین انزوتیک گوسفند، با موارد زیادی از مرگ و میر در مزارع گوسفند و بز در ایران همراه بوده است. با این حال، اطلاعات اپیدمیولوژیک کمی در مورد میزان سقط جنین مرتبط با کلامیدیا در ایران وجود دارد. بر این اساس، هدف ما بررسی میزان شیوع کلامیدیا آبورتوس و عوامل خطر مرتبط با آن در نشخوارکنندگان کوچک استان کرمان واقع در جنوب شرقی ایران بود. برای این منظور ۱۳۴ نمونه سواب واژینال از ۷۰ گوسفند و ۶۴ راس بزی که سقط جنین داشتند جمع آوری شد. پس از استخراج DNA از نمونه ها، ژن POMP90-3 کلامیدیا آبورتوس با استفاده از روش PCR برای تایید حضور این باکتری تکثیر داده شد. نتایج نشان داد که شیوع کلی کلامیدیا آبورتوس ۲۱/۶٪ است: ۲۰/۳ ٪ برای بزها و ۲۲/۸ ٪ برای گوسفند. میزان شیوع بیشتری در حیوانات با تعداد زایمان زیادتر مشاهده شد. با این حال، تفاوت معنی داری در میزان شیوع کلامیدیا آبورتوس در دو گونه حیوانی وجود نداشت. علاوه بر این، محل نمونه برداری به عنوان یک عامل خطر مرتبط با عفونت کلامیدیا آبورتوس در نظر گرفته شد. این مطالعه نشان داد که کلامیدیا آبورتوس به عنوان تهدیدی برای تولید مثل نشخوارکنندگان کوچک در استان کرمان محسوب می شود و ضرورت نظارت مستمر و اعمال راهبردهای پیشگیرانه را نشان داد.

واژگان کلیدی

PCR ،کلامیدیا آبورتوس، استان کرمان، نشخوارکنندگان کوچک

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Abstract in Persian

یک مورد گزارش آلودگی جغد شاخدار به ترماتود *Plagiorchis noblei* در شهرستان شهرکرد

نادر احمدی صالح بابری[1*] ، ریحانه قاسمی[2] ، نوید امامی[3] ، هاجر سهرابی نیا[3]

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چکیده

در سال ۱۴۰۱ از یک قطعه جغد شاخدار (*Asio otus*) در شهرستان شهرکرد ، برای اولین بار یک گونه خاص از ترماتود شناسایی شد . این ترماتود از روده باریک جغد شاخدار ، جداسازی شد . پس از رنگ آمیزی ترماتود با اسید کارمن، بر روی لام میکروسکوپی تثبیت شد و توسط استریومیکروسکوپ ، مورد بررسی قرار گرفت و با کلیدهای تشخیصی موجود شناسایی شد. مطالعات نشان می دهد که این ترماتود متعلق به جنس *Plagiorchis noblei* می باشد . تا به امروز حدود ۶۰۰۰ گونه از ترماتودهای دیژنه آ، توصیف شده اند . این انگل متعلق به خانواده *Plagiorchiidae* می باشد ولی تا کنون این انگل در شهرستان شهرکرد در جغد مشاهده نشده است و این گزارش، شرح آلودگی جغد شاخدار برای اولین بار به ترماتود *Plagiorchis noblei* در شهرستان شهرکرد می باشد. این انگل متعلق به راسته دیژنه آ می باشد که باعث ایجاد انواع بیماری های ریوی، گوارشی، کبدی و خونی در پرندگان و سایر مهره داران می شود. در ضمن، اطلاعات و داده های بیشتر درباره ی آلودگی موجودات یک اکوسیستم ما را در پیشگیری از عفونت ، بقا و پایداری موجودات آن اکوسیستم، یاری می دهد

واژگان کلیدی

ترماتود ، جغد شاخدار ، شهرکرد ، *Plagiorchiidae, Plagiorchis noblei*

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IRANIAN JOURNAL OF VETERINARY SCIENCE AND TECHNOLOGY

Author index

Guide for authors

SCOPE

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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.

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