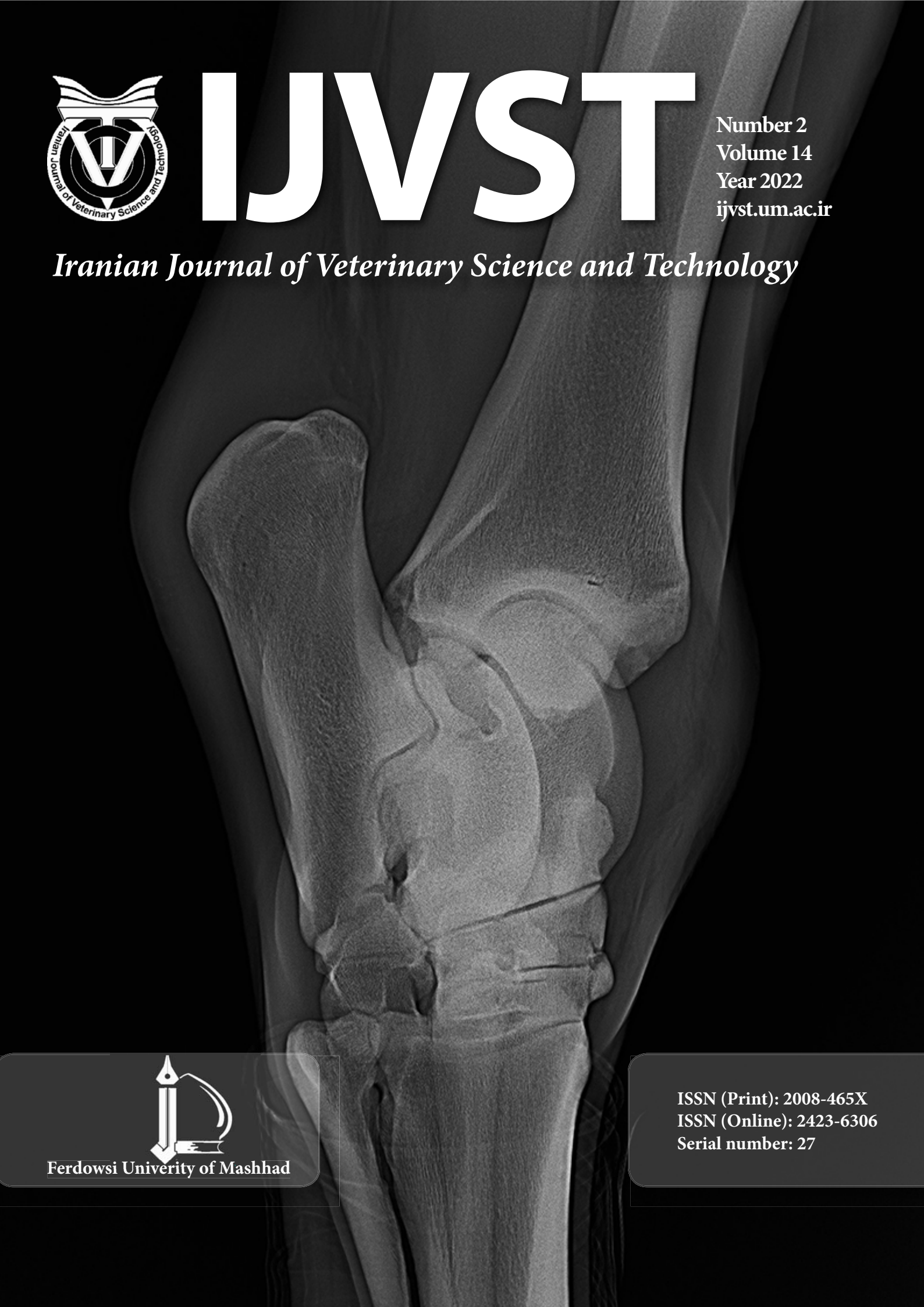

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ON THE COVER

A radiographic image of the tarsocrural joint in a 4-year-old English Thoroughbred showing synovitis; see page 47.

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A review on *Babesia* spp. and tick vectors in animals in Iran

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ABSTRACT

Babesiosis is an important tick-borne disease that affects a wide range of domestic and wild animals and occasionally humans in tropical and subtropical countries. So far, More than 100 different *Babesia* species have been identified in animals. Iran is one of the largest countries in the Middle East. The existence of two chains of mountains namely Zagros and Alborz has provided a number of climatic variations with different flora and fauna. These different climatic zones in Iran are potentially favorable for a large variety of tick vectors transmitting blood pathogen protozoa like *Babesia* spp. and *Theileria* spp. in animals. In the last decade, many molecular studies have been performed to identify *Babesia* spp. and tick vectors in different parts of Iran. This review article aims to provide useful information about the history, characters, geographical distribution, and prevalence of *Babesia* species and their related tick vectors in animals in Iran.

Keywords

Babesia spp., tick, animal, Iran

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Introduction

Babesiosis is an important tick-borne disease in domestic and wild animals. The causative agent and vectors for this disease are *Babesia* spp. and Ixodid ticks, respectively [1]. *Babesia* species live and multiply as 'piroplasms' in erythrocytes of the vertebrate host, and in contrast to erythrocytic stages of Plasmodium do not contain pigment [2]. The female tick usually is infected by ingesting protozoa in the blood meal and transmitting them to new hosts as sporozoites in the tick's saliva of the next generation, larvae, nymphs, and adults. The main clinical signs of babesiosis are fever, hemoglobinuria, anemia, and icterus [2]. Babesiosis has a wide geographical distribution in temperate, tropical, and subtropical regions. Iran is located in the western Palearctic countries with a high diversity of hard ticks [3] that could act as a vector for *Babesia* spp. in animals [3]. Advances in molecular biology methods have led to changes in the identification of *Babesia* species and their vectors in the world. Many molecular studies have been conducted on *Babesia* spp. identification in domestic animals in Iran from 1998 to 2015. Based on these results, two systematic reviews were also published about babesiosis in sheep, goats, cattle, and horses [4, 5]. This review provides useful information about the history, characters, geographical distribution, and prevalence of *Babesia* species, and their related tick vectors in animals in Iran.

History of Babesia

Babesia, also called *Nuttallia*, is an apicomplexan parasite that infects red blood cells and is transmitted by hard ticks. It was discovered in the red blood cells of cattle by the Romanian bacteriologist Victor Babes in 1888. He later observed a similar organism in sheep blood [6]. Five years later, Smith and Kilbourne showed the presence of an intraerythrocytic parasite in dairy cattle with Texas cattle fever, a disease that had long stricken cattle ranchers in the southern USA [7]. They were given the name *Pyrosoma bygeminum*, and showed that ticks play a major role in the transmission of this disease. This was the first description of an arthropod-transmitted, pathogen of vertebrates. Starcovici chose the name *Babesia* for these organisms in 1893 [8]. Lignieres described two forms of *Babesia* as *B. bigemina* and *B. bovis* in cattle in Argentina in 1903 [9]. In Iran, Delpy identified B. ovis in sheep for the first time in 1936 [10]. The first human babesiosis was reported in a splenectomized Yugoslavian farmer in 1957. After the initial case in Europe, a case caused by B. microti was diagnosed in a splenectomized patient from California, USA, in 1966. *Babesia crassa* as a large *Babesia* species was isolated for the first time in the world from an Iranian sheep in 1981 [13].

Taxonomy, transmission, and morphology

The genus *Babesia* belongs to the phylum *Apicomplexa*, and the family *Babesiidae*. *Babesia* is a relatively piriform, round, or oval parasite; the apical complex contains a polar ring, rhoptries, and subpellicular tubules. Micronemes and conoids are present in some stages and in some species [14]. Based on the merozoite size and comparison with erythrocyte radius, *Babesia* spp. are divided into large and small groups. The lengths of small and large *Babesia* are 1.0 to 2.5 µm and 2.5 to 5.0 µm, respectively. The morphometric method has no clear genetic basis, because the size and morphology of *Babesia* spp. may be changed during the asexual stage within red blood cells or when infects a non-specific host [15, 16]. So far, over 100 *Babesia* species have been identified in vertebrate hosts. Of those, eighteen species have been found to cause babesiosis in domestic mammals, including pigs, horses, cattle, sheep, goats, cats, and dogs. Most *Babesia* species have been reported in rodents, cattle, and carnivores (Table 1).

Life cycle

The life cycle of *Babesia* spp. consists of at least the asexual and sexual stages of reproduction that occur within the vertebrate host and tick vector, respectively. The sporozoites of the tick's salivary glands are generally transmitted to the vertebrate host 2-3 days after tick attachment. The sporozoites change to merozoites and enter red blood cells and divide by binary fission into new merozoites. Infected erythrocytes eventually rupture and release organisms that invade and multiply within other red blood cells. Some of the merozoites become pre-gametocytes that cannot be distinguished by a light microscope. When the tick vectors ingest the infected blood of the vertebrate host, the merozoites are microscopically detectable in the tick's gut after 10 hours [16]. The pre-gametocytes develop into gametocytes and begin to form ray bodies at the anterior of the piroplasm. The ray bodies form gametes and fuse to produce a motile zygote termed ookinete, which enters the gut epithelium cells. The ookinete starts meiotic division, resulting in many kinetes productions. At this stage, the kinetes migrate via hemolymph to different tick tissues such as ovarian cells. The infection of eggs leads to transovarial transmission. Some kinetes enter the salivary gland cells where a large multinuclear sporont is finally formed, giving rise to thousands of small sporozoites, which are injected during the feeding act and lead to transstadial transmission (Figure 1) [17, 18].

Babesia spp. infection in domestic animals in Iran

Cattle

Table 1.
Different *Babesia* species and tick vectors with geographical distribution in domestic animals [1]

Host	Species	Morphology	Tick vector	Distribution
Cattle	*B. bovis*	Small	Boophilus,Rhipicephalus	Africa, America, Asia, Australia, Europe
	B. Bigemina	Large	Boophilus,Rhipicephalus	Africa, America, Asia, Australia, Europe
	B. major	Large	Haemaphysalis	Asia, Europe
	B. occultans	Large	Hyalomma	Africa
	B. ovata	Large	Haemaphysalis	Asia
	B. divergens	Large	Ixodes,	Europe
	B. sp. Kashi	Large	Hyalomma	China
Buffalo	*B. orientalis*	Small	Rhipicephalus	Asia
	B. bovis	Small	Boophilus,Rhipicephalus	Asia, America
	B. bigemina	Large	Boophilus,Rhipicephalus	Asia, America
Horse, Donkey	*B. equi*	Small		Asia, Europe, America
	B. caballi	Large	Dermacentor, Hyalomma, Rhipicephalus	Asia, Europe, America
Pig	*B. trautammani*		Rhipicephalus	Africa, Europe
Sheep, Goat	*B. ovis*	Small	Rhipicephalus	Africa, Asia, Europe
	B. motasi	Large	Haemaphysalis	Africa, Asia, Europe
	B. crassa	Small	Unknown	Asia
Dog	*B. vogeli*	Large	Rhipicephalus sanguineus	Africa, America, Asia, Australia, Europe
	B. conradae	Small		America
	B. gibsoni	Small	Haemaphysalis longicornis, Rhipicephalus	Africa, America, Asia, Australia, Europe
	B. rossi	Large	Haemaphysalis	South Africa
	B. canis	Large	Dermacentor	Europe
Cat	*B. felis*	Small	Unknow	South Africa
	B. cati		Unknow	India

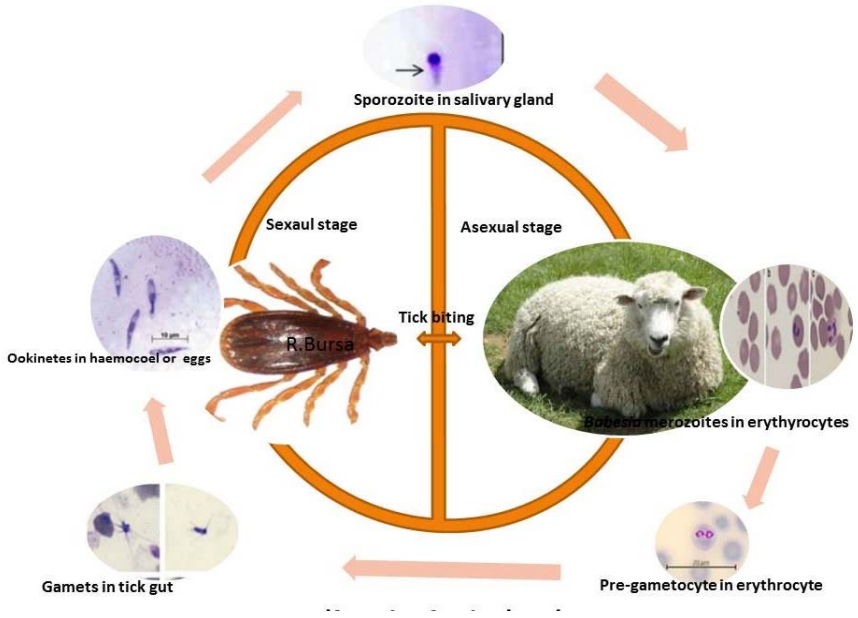

Figure 1.
The life cycle of *Babesia ovis* [1].

In Iran, the main species of *Babesia* in cattle are *B. bigemina* and *B. bovis* [19]. The first outbreak of babesiosis due to *B. bovis* was reported from a dairy farm in the Rasht area [20]. *B. bovis* localizes near the margin of the erythrocyte and is clearly smaller than B. bigemina and larger than *Theileria annulata*. The shape of *B. bovis* is ring form or ovoid. The erythrocytic stage of *B. bigemina* is large, round, oval, or pear-shaped and fills the whole erythrocyte when divided [17]. Few studies have been performed on bovine babesiosis compared to ovine babesiosis in Iran. It seems that *B. bovis* and *B. bigemina* are more common in cattle in western and northwestern Iran (Table 2). Among Ixodid ticks, *B. annulatus*, *R. Bursa*, and *R. sanguineous* could be the vector for *Babesia* spp. in dairy cattle [23, 24]. The large *B. occultance* was recently reported from two cattle in the Miandoab area by molecular methods [27].

Table 2.
Reported *Babesia* species and related tick vectors in cattle in Iran

Year	*Babesia* species	Tick vector	Province or Area	Prevalence	Diagnostic methods	References
1977	*B. bovis*	*B. annulatus*	Rasht	-	Microscopic examination	20
2012	B. bigemina	-	Tabriz	-	Microscopic examination, PCR	21
2012	B. bigemina		Kurdestan	21%	Microscopic examination	22
2012	*Babesia* spp.	*R.sanguineus* and *R. bursa*	*Kurdestan and West Azarbijan*		PCR	23
2017	B. bigemina B. bovis	B.annulatus R.sanguienus R.bursa	Urmia	42%	Microscopic examination, PCR	24
2020	B.bigemina B.bovis	-	East and West Azarbijan	25.49%	Microscopic examination, PCR	25
2020	B. bigemina B. bovis	-	Mazandaran	33.33%	PCR	26
2021	B. occultans	-	Miandoab	-	Microscopic examination, PCR	27

Sheep and goats

Three *Babesia* species including *B. ovis, B. motasi,* and *B. crassa* have been reported in infected sheep and goats in Iran (Table 3). *Babesia ovis* is a small round piroplasm, situated usually at the periphery of the red blood cells of infected sheep [17]. This species is widespread in almost all parts of Iran [28, 29]. *Babesia motasi*, as a large species is less prevalent in Iran [30]. *Babesia ovis* is high pathogenic and causes anemia and hemoglobinuria, while *B. motasi* appears moderately virulent [31]. *Babesia crassa* is a large species that was isolated from an Iranian sheep. It is characterized by an oval tetrad form in infected erythrocytes. The protozoon appears to be nonpathogenic to intact sheep and goats [13]. The outbreaks of ovine babesiosis are recorded in sheep and goats at the age of 6- 12 months each year [32-33]. Potential vectors for *B. ovis* could be *R. bursa, R. sanguineous,* and *R. turanicus* [35, 36]

Horse and donkey

Babesia equi and *B. cablli* were reported from horses in different areas of Iran (Table 4). Studies have shown that *B. equi* is more prevalent than *B. caballi* in Iranian horses and donkeys. The presence of *B. caballi* and *T. equi* was confirmed in 1940 by microscopic and molecular examination [54]. A few case reports of babesiosis due to *B. caballi* and *B. equi* have been published on horses in different parts of Iran from 1994 to 2000 [55-58]. In a study, *B. equi* infection was determined in donkeys of North Khorasan province by microscopic and molecular methods [67]. The name of *B. equi* has recently been changed to *Theileria equi*, because the sporozoites of *T. equi* first evade the lymphocytes and multiply by schizogony. After rupture of infected lymphocytes, the released merozytes enter the red blood cells and change to rounded, amoeboid, and a Maltese cross-shaped phenotype [2]. The vectors of *T. equi* could be *Hyalomma* spp. and also *Rhipicephalus* spp.. The eggs of these ticks were not infected with ookinetes to indicate transovarial transmission [17]. The merozoites of *B. caballi* are large and pear-shaped. They are produced by binary fission. *Babesia* infections are always detected in the eggs of tick vectors that could be transmitted to larvae in the next generation [17].

Table 3.
Reported Babesia species and related tick vectors in sheep and goats in Iran

Year	*Babesia* species	Tick vector	Province or Area	Prevalence	Diagnostic methods	References
1936	*B. ovis*	-	-	-	Microscopic examination	10
1966	B. motasi	-	West of Iran	-	Microscopic examination	32
1981	B. crassa	-	-	-	Microscopic examination	13
1998	*B. ovis*	-	Caspian sea, Mountainous Persian gulf Desert climates	15.93% *58.81%* *12.04%* *13.22%*	Serology	34
2002	B. ovis B. motasi	R.sangueineus Hy.marginatum	Mashhad	*24.6%* o.5%	Microscopic examination	35
2003	B. ovis B. motasi	-	Mashhad	*14%, 0.5%*	Microscopic examination	36
2006	B. ovis		Khouzestan	*47.5%*	Serology	37
2006	B. ovis B. motasi	-		-	Microscopic examination, PCR	38
2007	B.ovis	R.bursa R.sangueinus, R.turanicus			PCR	39
2008	B. ovis	-			PCR	40
2010	B. ovis		Different areas of Iran	*24.6%*	Microscopic examination, PCR	41
2012	B. ovis	-	-	-	Reverse line blot	42
2013	B. ovis	-	Tabriz	*14%*	PCR	43
2013	B. ovis		Dargaz, Kalat	*0.99%*	Microscopic examination, PCR	44
2013	B. ovis B. motasi	-	Mazandarn province	*5%*	Microscopic examination, PCR	45
2014	B. ovis	*R. turanicus, Hya. marginatum*	North khorsan province		Microscopic examination, PCR	46
2014	B. ovis	R. bursa	West Azarbijana	*16.7%*	Microscopic examination, PCR	47
2017	B. ovis	D. niveus| D. marginatus	Ardabil		PCR	48
2017	*B. ovis*	-	Lorestan		PCR	49
2017	B. ovis	R. sanguienus Hya. suspense	Gonbad Kavoos, Marvaeh tapaeh	-	PCR	50
2018	*B. ovis*		East azerbijan	*11.04%*	PCR	51
2020	*B. ovis*		Baneh	*86%*	PCR	52
2020	*B. ovis* *B. motasi*	-	Tonkabon, Ramsar	*6%*	PCR	53

Table 4.
Reported *Babesia* species and related tick vectors in horses and donkeys in Iran

Year	*Babesia* species	Tick vector	Province or Area	Prevalence	Diagnostic methods	References
2000	*B. cabali*	-	Fars province	-	Microscopic examination	57
2000	B. cabali, B. equi	-	Mashhad	-	Microscopic examination	58
2013	B.cabali, B.equi		North Khorasan Province	2%, 48%	Seology	59
2014	B. equi	*Hya. excavatum, Rh. bursa*	North Khorasan Province	45%	Microscopic examination, serology , PCR	60
2014	*B. cabali*		North Khorasan Province	4.8%	Microscopic examination, Serology	61
2014	*B. cabali, B. equi*		Urmia area	2.08%, 26%	Microscopic examination, PCR	62
2014	*B. equi*	-	Khuzestan Province	28.5%	PCR	63
2014	B. equi		Yazd area	4.7%, 22.8%	Microscopic examination, PCR	64
2014	B. equi	-	Mianeh area	4.1%	Microscopic examination	65
2015	B. equi	-	Ahvaz area		Microscopic examination,PCR	66
2015	B. equi	-	North Khorasan Province	3.77%, 50.94%	Microscopic examination,PCR	67
2016	B. equi		Piranshar area	9.6%,96%	Microscopic examination , PCR	68
2017	B. equi, B. caballi	-	Isfhan, Sharekord	-	PCR	69
2017	B. equi	-	Kurdistan	1.61%	Microscopic examination , PCR	70
2018	B. equi	-	West Azarbijan	3.2%, 27.7%	Microscopic examination , PCR	71

Dog

For the first time, *Babesia* canis, and *B. gibsoni* were reported in the blood smear of splenectomized dogs and foxes from the north of Iran in 1973 [72]. Further study was shown that the isolated strain is mild and does not produce clinical signs in experimentally infected dogs. Many studies have reported the large *Babesia* spp. in dogs of different parts of Iran (Table 5). *Babesia canis* as a large *Babesia* has three subspecies, *B. canis vogeli*, *B. canis rossi*, and *B. canis canis*. They are different in genotype, geographic distribution, pathogenicity, and vector-specificity [73].

Table 5.
Reported *Babesia* species in dogs in Iran

Year	*Babesia* species	Tick vector	Province or Area	Prevalence	Diagnostic methods	References
1973	*Babesia canis* *Babesia gibsoni*	-	Mazandaran Province	0.64%	Microscopic examination	72
2012	B. canis		Shiraz	-	PCR	74
2013	B. canis		Khousestan Province	3.75%	Microscopic examination	75
2014	B. canis		Charmahal Bakhtiri	7.5%	PCR	76
2016	*B. gibsoni*		Kerman province	5%	PCR	77
2020	*B. canis vogeli*		Shariar	-	PCR	78
2021	*B. canis vogeli*		Hamadan	4%	PCR	79
2022	B. canis canis		Tehran	-	PCR	80

Babesia gibsoni, B. conradea, and *Theileria annae* are termed small canine *Babesia*. Among different hard tick species, it has been reported that *R. sanguineous* could act as a vector for *B. vogeli* and *B. canis*, and *Haemaphysalis spp.* as a veorct for *B. rossi* and *B. gibsoni* [73].

Camel

So far, a specific *Babesia* species has not been reported in camels worldwide. Based on molecular methods, *Babesia* species related to cattle and horses have been found in camels [81]. *Babesia caballi* and *T. equi* have been detected in camels in Iran [82-84].

Rodents

Babesia microti, a species of rodent origin, has been recognized as an agent of human babesiosis in the world [1]. There are a few reports about the presence of *Babesia microti* in Iran [85-87].

Conclusion

This review presented a comprehensive summary of research findings on the identification, prevalence, and distribution of *Babesia* species and their related vectors in domestic animals in Iran. In the last decade, many molecular studies have been performed to identify *Babesia* spp. and tick vectors in different parts of Iran. However, there is no information about *Babesia* infection in cats and wild animals. Further molecular and experimental methods will be needed to better understand the epidemiology of *Babesia* species and their related tick vectors in domestic and wild animals.

Competing Interests

The authors declare that they have no conflict of interest.

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Prevalence assessment of *Salmonella* serovars in apparently healthy pet dogs in Tehran, Iran

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ABSTRACT

Salmonellosis is considered to be a zoonotic disease, the transmission of which through oral-fecal contact is unavoidable because pet care has been popular recently. On the other hand, excessive use of human antibiotics to treat animals resulted in the emergence of antibiotic-resistant *Salmonella* serotypes. This study aimed to assess the prevalence of bacteria and antibiotic resistance to select the appropriate antibiotic for disease control. In this study, the presence of *Salmonella* serovars in the fecal samples of 256 pet dogs was investigated by enrichment and selective culture. Moreover, the existence of virulence and antibiotic resistance genes, as well as phenotypic antimicrobial resistance, were assessed. Of the total of 256 fecal samples, 21 samples (8.2%) of pet dogs were positive for *Salmonella*, including S. *Typhimurium*, S. *Enteritidis*, S. *Infantis*, and S. *Senftenberg*. Based on our findings, all serovars carried virulence genes *invA, invF, sitC, fimA* and *S. Typhimurium* resistant to ampicillin (100%), tetracycline (50%), oxytetracycline (75%), florfenicol (50%) and lincospectin (100%). While *S. enteritidis*, *S. infantis,* and *S. senftenberg* were sensitive to ampicillin, amikacin, gentamicin, and ciprofloxacin. *S. Infantis* was also sensitive to all antibiotics. In conclusion, our findings suggest that pet dogs are potential sources of *Salmonella* strains that carry resistance and virulence genes. Thus, healthy pet dogs could play an important role in human salmonellosis.

Keywords

antibiotic resistance gene, pet dog, Salmonella, virulence gene

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Abbreviations

S. Typhimurium: Salmonella typhimurium
S. Infantis: Salmonella infantis
S. Enteritidis: Salmonella enteritidis
S. Senftenberg: Salmonella senftenberg

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Introduction

In developed countries, dogs are one of the most popular pet animals and the relationship between humans and pets has changed dramatically [1]. Direct contact of dogs and humans with food and feces transmits bacteria to humans that pose a greater potential risk to children than adults [2]. One of the most important zoonotic bacteria is *Salmonella* [2, 3]. *Salmonella* is a gram-negative bacterium that includes endotoxins, enterotoxins, siderophores, flagella, and virulence plasmids. In humans and animals, this bacterium can cause gastroenteritis, pneumonia, abortion, and lethal sepsis. The *Salmonella* genus contains more than 2659 serovars [4].

Dogs are generally resistant to infection and and serve as carriers for human salmonellosis without any clinical symptoms. Indeed, most of antibiotic resistance genes identified in human infection are correlated with dog salmonellosis [5, 6]. On the other hand, pet dogs could be an important source of antibiotic-resistant serovars. Therefore, these animals are considered a public health, particularly for children, the elderly, and immunocompromised individuals [7-9].

Salmonella serovar prevalence in dogs is influenced by several variables: First, environment, if they contact wild animals or infected animals. Second, raw food was already reported to have a high-risk factor in *Salmonella* serovars prevalence. Third, in microbiota alteration, normal microbiota could inhibit the gut tract from pathogen colonization while microbiota change can provide an environment for pathogen replacement [3, 10, 11].

Generally, *Salmonella* virulence factors, such as the invA, invF, sitC, and fimA, are chromosomal, while antibiotic resistance genes are located on plasmids. For example, β-lactams, aminoglycosides, tetracyclines, trimethoprim, and sulfonamide resistance-related genes (*blaCMY-2, blaCMY-9, aac(3)-Ia, aac(3)-IIa, tetA, tetB, dhfrI, dhfrII, sulI, sulII*) [12-15].

Salmonella is of high importance in public health and human diseases. Furthermore, the desire to have pet dogs is increasing in Iran. however, no recent research has evaluated the prevalence of *Salmonella* serovars in healthy dogs in Iran.

Therefore, the aim of this study was the assessment of the presence of *Salmonella* serovars in healthy pet dogs in Tehran, Iran. Moreover, the virulence factors and antibiotic resistance genes (mentioned above) were also evaluated.

Results

The prevalence of Salmonella serovars in healthy pet dogs

Isolation of *Salmonella* serovars was confirmed based on the cultural and biochemical methods. Out of the specimens collected from 256 dogs, 21 samples (8.2%) were positive for *Salmonella* (17 samples from Tehran University Veterinary Hospital and 4 samples from Khavarmiane Veterinary Hospital).

Isolated *Salmonella* serovars were serotyped with O and H antisera. Serotyping revealed four differents serovars: *S. Typhimurium* (n = 4); *S. Infantis* (n = 4); *S. Enteritidis* (n = 10) and *S. Senftenberg* (n = 3) (Table 1).

Detection of Salmonella virulence genes

The results of PCR amplification of the extracted DNA from 21 isolates on *invA, invF, sitC,* and *fimA* virulence genes showed that all samples (100%) had *invA* gene. Moreover, *invF, sitC,* and *fimA* genes were detected in 19 samples (90.47%). All virulence genes were detected in *S. Typhimurium* and *S. Infantis* (Table 2). All the samples of *S. enteritidis* serovar showed all virulence genes except one which was *sitC*-negative. In the *S. Senftenberg* serovar, two isolates were positive for *sitC* and one was positive for *invF* and *fimA* virulence genes (Table 3).

Antibiotic resistance genotype

The results of the detection of antibiotic resistance genes are shown in Table 4. The prevalence of antibiotic resistance genes was examined in different strains. All isolates of *S. Typhimurium* were positive (100%) for *blaCMY-2, tet A,* and *sul I*. For the other genes, fewer isolates were positive. Furthermore, in *S. infantis*, the most prevalent resistance genes were *blaCMY-2, aac(3)-Ia, dhfrI, sul II,* and *tet A*, while the least prevalent genes were *sulI, dhfr II, tet B,* and *aac(3)-IIa*. In *S. enteritidis*, the most prevalent resistance genes included *aac(3)-IIa, tet B,* and *dhfrII*, while *tetA* and *aac(3)-Ia*

Table 1.
Salmonella serovars isolated from dogs (n=21)

Salmonella Serovar	serogroup	H1	H2	number	percentage (%)
Salmonella typhimurium	B (1,4,5,12)	i	1,2	4	19.04
Salmonella infantis	C1(6,7)	b	1,2	4	19.04
Salmonella enteritidis	D (1,9,12)	g.m	---	10	47.61
Salmonella senftenberg	E4(1,3,19)	g.s.t	---	3	14.28
Total				21	

Table 2.
Distribution of the virulence genes (n=21)

Virulence gene	number	percentage (%)
invA	21	100
invF	19	90.47
SitC	19	90.47
fimA	19	90.47

Table 3.
Presence of virulence genes in *Salmonella serovars*

Salmonella Serovars	virulence genes (%)			
	invA	invF	sitC	fimA
Salmonella typhimurium	4 (100%)	4 (100%)	4 (100%)	4 (100%)
Salmonella infantis	4 (100%)	4 (100%)	4 (100%)	4 (100%)
Salmonella enteritidis	10 (100%)	10 (100%)	9 (90%)	10 (100%)
Salmonella senftenberg	3 (100%)	1 (33.33%)	2 (66.66%)	1(33.33%)

had a low prevalence. The lowest abundance of *blaCMY-2, blaCMY-9, aac(3)-IIa, tet B, dhfrI,* and *sulII* genes were detected in *S. Septenberg.*

Antimicrobial resistance phenotypes

According to the results, *S. Typhimurium* was resistant to ampicillin (100%), Tetracycline (50%), Oxytetracycline (75%), Florfenicol (50%), and Lincospectin (100%). On the other hand, all isolates belonging to *S. Enteritidis, S. Infantis* and, *S. Senftenberg* were sensitive to Ampicillin, Amikacin, Gentamicin, and Ciprofloxacin. *S. Infantis* was also sensitive to all antibiotics (Table 5).

Table 4.
Distribution of the antimicrobial resistance genes in *Salmonella* serovars

Salmonella Serovars	Antimicrobial resistance genes									
	blaCMY-2	blaCMY-9	*aac(3)-Ia*	*aac(3)-IIa*	*tetA*	*tetB*	*dhfr I*	*dhfr II*	*Sul I*	*Sul II*
Salmonella Typhimurium	4(100%)	3(75%)	3(75%)	0(0%)	4(100%)	2(50%)	3(75%)	1(25%)	4(100%)	0(0%)
Salmonella Infantis	4(100%)	2(50%)	3(75%)	1(25%)	3(75%)	2(50%)	4(100%)	1(25%)	2(50%)	3(75%)
Salmonella Enteritidis	9(90%)	6(60%)	6(60%)	4(40%)	5(50%)	6(60%)	7(70%)	4(40%)	6(60%)	9(90%)
Salmonella Senftenberg	0(0%)	0(0 %)	2(66.6%)	0(0 %)	2(66.6%)	1(33.3%)	2(66.6%)	1(33.3%)	2(66.6%)	2(66.6%)

Discussion

Salmonella is one of the main causes of food poisoning, diarrhea, and gastroenteritis in humans [16]. Acute gastroenteritis is one of the most prevalent diseases in regions with low public health [17]. Salmonellosis is known as a common disease between humans and animals. Since keeping pets, especially dogs has become popular in recent years, the possibility of disease transmission through regular contact with feces (fecal-oral transmission) of animals is inevitable. In Iran, a significant percentage of gastroenteritis in children is related to *Salmonella* [18-20]. In recent years, the incidence of non-typhoid *Salmonella* has increased dramatically due to the emergence of many *Salmonella* serotypes [21, 22].

The *Salmonella* serovars have been isolated from 0%-79% of healthy pet dogs in diverse regions of the world [5, 6, 23, 24]. There are few studies on the infection of dogs with *Salmonella* in Iran. The first study in Tehran on outdoor dogs was carried out by Shimi et al. in 1976, it was shown that 15.8 % of dogs were infected with the serotypes of *Salmonella Derby* and *Newport* [25]. Zahraei Salehi et al. in 2013 found that 10.5% of dogs in Garmsar region were infected with *S. Reading* serotype [26]. Nimrodi et al. investigated dog feces specimens from ten rural areas of Mazandaran, Iran, and reported that 50%, 35%, and 15% of the isolates were *S. Enteritidis, S. Typhimurium*, and *S. Dublin*, respectively. The most frequent serovar in the latter study was *S. Enteritidis* [27]. In the present study, the prevalence of *Salmonella* serovars was 8.2% in Tehran. Four serovars were isolated, with *S. Enteritidis* (47.61%) and *S. Typhimurium* (19.04%) predominating as the major serovars associated with human disease. This difference in the prevalence of *Salmonella* first can be due to geographical variation [5, 23, 28] and then differences in the sample sizes, fe-

Table 5.
Antimicrobial resistance /susceptibility phenotypes of isolated *Salmonella* serovars

Antimicrobials	strains	S. Typhimurium			S. Infantis			S. Enteritidis			S. Senftenberg		
	No. of strains	4			4			10			3		
		S	I	R	S	I	R	S	I	R	S	I	R
AM		0	0	4	4	0	0	10	0	0	3	0	0
FOX		4	0	0	4	0	0	10	0	0	3	0	0
CPM		4	0	0	4	0	0	10	0	0	3	0	0
CEF		4	0	0	4	0	0	10	0	0	3	0	0
GEN		4	0	0	4	0	0	10	0	0	3	0	0
AMK		4	0	0	4	0	0	10	0	0	3	0	0
T		1	1	2	2	2	0	9	0	0	0	0	3
OTC		0	1	3	4	0	0	0	1	9	0	0	3
DOX		3	1	0	4	0	0	9	1	0	1	2	0
FLO		0	2	2	4	0	0	10	0	0	1	0	2
LS		0	0	4	4	0	0	9	0	1	3	0	0
ENR		4	0	0	4	0	0	10	0	0	3	0	0
CIP		4	0	0	4	0	0	10	0	0	3	0	0
TS		4	0	0	4	0	0	9	0	1	2	0	1

S denotes susceptible, I denotes intermediate resistance, and R denotes resistance. Antimicrobials: AM (ampicillin), FOX (Cefoxitin), CPM (cefepime), CEF (Ceftiofur), GEN (Gentamicin), AMK (Amikacin), T (Tetracycline), OTC (Oxytetracycline), DOX (Doxycycline), FLO (Florfenicol), LS (Lincospectin), ENR (Enrofloxacin), CIP (Ciprofloxacin), TS (Trimethoprim-Sulfamethoxazole).

cal sampling conditions, and isolation and detection methods employed. There have been many reports of different *Salmonella* serotypes being isolated worldwide from the feces of healthy dogs. About 53 serotypes were isolated, most of which were related to *S. Typhimurium, S. Anatum, S. Panama, S. Krfeld, S. Bronx, S. Newport, S. Indiana, S. Kentucky, S. Saintpaul,* and *S. Virchow* [29, 30]. Unlike developing countries where the pet dogs are fed a commercial diet, the main dog food in Iran is cooked homemade food, such as rice and chicken. Nadi, et al. in a study on 1425 stool samples (obtained from *Salmonella* outbreaks, 2013-2019) revealed that *S. Enteritidis* and *S. Senftenberg* were major Salmonellosis agents in Iran with frequencies of 26.3% and 21.3%, respectively [31]. A study conducted by Chantharothaiphaichit that healthy household dogs multidrug-resistant *Salmonella Enterica* [32].

In the world, as well as in Iran, *S. Enteritidis* is the major salmonellosis agent with the food source [33, 34]. Also, several studies have shown that a Salmonellosis agent was detected in cooked poultry and cooked meat [35, 36]. According to previous studies, food is one of the main sources of *Salmonella* infection in pet dogs, which can infect humans. Moreover, in our research, all isolates were positive for *invA* virulence gene. This gene is an international standard for identifying *Salmonella* (Malorny, Hoorfar, Bunge, & Helmuth, 2003). A previous study in Iran reported that the frequency of virulence genes in 13 positive *Salmonella* samples was reported as follows: *invA* (100%), *invF* (23.1%), and *sitC* (0%). However, due to the lack of serotyping, these results are not reliable [13]. In England and Iraq, all isolates carried *sitC* and *fimA* [37,38] which is consistent with our finding.

Diarrhea is the most common symptom of human salmonellosis [39,40]. Therefore, the assessment of antibiotic resistance to *Salmonella* serovars in dogs is especially important. The genotype and phenotype of antibiotic resistance of serovars have been investigated in our research. All isolates of *S. Typhimurium* were resistant to third-generation Ampicillin. We also found that *S. Typhimurium, S. Enteritidis,* and *S. Senftenberg* were resistant to the Tetracycline group except for *S. Infantis*. Several studies have shown that Tetracycline/Oxytetracycline resistance in *Salmonella* serovars is common [40,41]. Fortunately, The first antibiotic choice for non-typhoid salmonellosis in humans is ciprofloxacin [42], to which all isolates were susceptible in the present study. Similar results were

reported with our study on *Salmonella* isolates from around the world [31, 43-45]. In conclusion, in the present study, it was shown that *S. Enteritidis*,*S. Typhimurium*, *S. Infantis*, and *S. Senftenberg* are the main serovars respectively in apparently healthy pet dogs in Tehran. The prevalence of *Salmonella* in the feces of pet dogs was evaluated to be 8.2%. Food is a possible contamination source in dogs. Isolated serovars have the potential to cause infection in humans. In this study, despite resistance to some antibiotic susceptibility to antimicrobials of choice for the treatment of human salmonellosis detected. Thus, our finding provided promising information on the prevalence of *Salmonella* serovars and their antibiotic resistance in pet dogs which can contaminate their owners. Regular monitoring of pet dogs can play an important role in controlling human Salmonellosis.

Materials & Methods

Sample collection

All animals were handled according to animal care rules of the Faculty of Veterinary Medicine, University of Tehran, Tehran. In this study, we used 206 fecal samples of pet dogs (age under 4 years, during 2000-2001) collected from the small animal hospital of Teheran University and 50 samples (age under 4 years, during 2020-2021) from Khavarmiane Veterinary Hospital, Tehran. The health conditions of the animals were checked and they did not show any specific symptoms of the disease. Rectal swabs were collected and transported under refrigeration to the microbiology laboratory of the Faculty of Veterinary Medicine, University of Tehran.

Salmonella serovars isolation and serotyping

Salmonella isolation was using a standard method (ISO 6579: 2002). Briefly, each rectal swab was enriched for 24 h at 37 °C in 1:10 vol/vol buffered Peptone water 2.5% (Merck, Germany). Then, 100 µl of the culture suspension was spotted on MacConkey agar (Merck, Germany) and incubated at 37 °C for 24 h. Next, Colonies were selected for inoculation onto Salmonella Shigella agar (SS agar, Merck, Germany) at 37 °C for 24 h. *Salmonella* suspicious colonies were biochemically confirmed by applying oxidase and catalase tests, triple sugar iron agar (TSI) test and IMViC group tests. After biochemical confirmation, the isolates were serotyped by specific antisera according to the manufacturer's instructions (BD Difco, USA).

DNA extraction

The *Salmonella* serovars DNA was extracted via the boiling method and the DNA samples were stored at -20 °C until analysis [46].

Primers

In this study, 14 primers were purchased from the Sina Clone company (Tehran, Iran). Four virulence-related genes, including invA, invF, sitC, and fimA (Table 6) and ten antibiotic resistance genes were examined and confirmed at NCBI and Primer-BLAST sites (Table 7).

Conventional PCR Assays

The PCR was run in 25 µl reaction mixture using the PCR master mix (Amplicon, Denmark). A total volume of 25 µl of reaction mixture contained 1µM primer, 3 µl template DNA, 7.5 µl sterile distilled water, and 12.5 µl master mix. Initial denaturation for detecting *invA, invF, sitC,* and *blaCMY-9* genes was performed at 94 °C for 5 min followed by 34 cycles of amplification. The amplification cycle included the following 3 steps: 94 °C for 1 min (denaturation), 60 °C for 1 min (annealing), and 72 °C for 1 min (extension). The polymerase chain reaction for other genes was similar to the previous steps except that the annealing temperatures for *fimA, aac(3)-Ia, dhfrI,* and *dhfrII* genes was 55 °C for 1 min, for *blaCMY-2* was 56 °C for 1 min, *aac(3)-IIa* 52 °C for 1 min, and for *tetA, tetB, sulI*, and *sulII* genes was 72 °C for 1 min. After 34 amplification cycles, the samples were retained at 72 °C for 5 min to ensure complete strand extension. The standard strain of *Salmonella* (microbial collection of the Faculty of Veterinary Medicine, Tehran university) was used as positive control and distilled water was used as negative control.

PCR Product analysis

Analyzing the PCR products completed by using 1% agarose gel-stained with 0.5 µg/mL ethidium bromide. The PCR products were visualized by a UV transilluminator and photographed using a digital camera.

Antibiotic susceptibility

The antibiotic susceptibility of all isolates was tested according to the Clinical and Laboratory Standards Institute protocols [47]. The antibiotics selected to test Salmonella serovars. include Ampicillin (10 µg), Cefoxitin (30 µg), Cefepime (30 µg), Ceftiofur (30 µg), Gentamicin (10 µg), Amikacin (30 µg), Tetracycline (30 µg), Oxytetracycline (30 µg),

Table 6.
Primers used for the detection of *Salmonella* virulence genes

Virulence factor	Target virulence gene	Sequence 5' to 3'	Product size (bp)	References
Invasion factor F	*inv*F	F: AAGGGATCCATGTCATTTTCTGAAAGCGACAC R: GTTGTAGGGAAAGCTTCTCCAGTAATG	918	[13]
Invasion factor A	*inv*A	F: GTG AAA TTA TCG CCA CGT TCG GGC AA R: TCA TCG CAC CGT CAA AGG AAC C	284	[35]
Salmonella iron transporter C	*sit*C	F: CAGTATATGCTCAACGCGATGTGGGTCTCC R: CGGGGCGAAAATAAAGGCTGTGATGAAC	250	[13]
fimbrial protein A	*fim*A	F: CCT TTC TCC ATC GTC CTG AA R: TGG TGT TAT CTG CCT GAC CA	85	[35]

Doxycycline (30 µg), Florfenicol (30 µg), Lincospectin (100 µg), Enrofloxacin (5 µg), Ciprofloxacin (5 µg), and Trimethoprim-Sulfamethoxazole (240+52 µg). The antibiotic discs were purchased from Padtan Teb company, and zone diameters were assessed and categorized as susceptible, intermediate, or resistant according to company guideline tables.

Table 7.
Primers used for the detection of antibacterial resistance genes in *Salmonella serovars*

Antimicrobial agent	Target resistance gene	Sequence 5' to 3'	Product size (bp)	References
β-lactam	*blaCMY-2*	F: TGGCCGTTGCCGTTATCTAC R: CCCGTTTTATGCACCCATGA	870	[13]
	blaCMY-9	F: TCAGCGAGCAGACCCTGTTC R: CTGGCCGGGATGGGATAGTT	847	
Aminoglycoside	*aac(3)-Ia*	F: TGAGGGCTGCTCTTGATCTT R: ATCTCGGCTTGAACGAATTG	436	[13]
	aac(3)-IIa	F: CGGCCTGCTGAATCAGTTTC R: AAAGCCCACGACACCTTCTC	439	
Tetracycline	*tetA*	F: GCGCCTTTCCTTTGGGTTCT R: CCACCCGTTCCACGTTGTTA	831	[13]
	tetB	F: CCCAGTGCTGTTGTTGTCAT R: CCACCACCAGCCAATAAAAT	723	
Trimethoprim	*dhfrI*	F: CGGTCGTAACACGTTCAAGT R: CTGGGGATTTCAGGAAAGTA	220	[13]
	dhfrII	F: AGTTTGCGCTTCCCCTGAGT R: CTTAGGCCACACGTTCAAGTG	194	
Sulfonamide	*sulI*	F: TCACCGAGGACTCCTTCTTC R: CAGTCCGCCTCAGCAATATC	331	[13]
	sulII	F: CCTGTTTCGTCCGACACAGA R: GAAGCGCAGCCGCAATTCAT	435	

Authors' Contributions

AAK, RY, and TZS conceived and designed research. AAK, RY, TZS, IAT, and BB conducted experiments. AAK, BB, RY, and TZS analyzed data. AAK and BB wrote the manuscript. RY and TZS edited the manuscript. All authors read and approved the manuscript.

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Competing Interests

The authors declare that they have no conflict of interest.

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RESEARCH ARTICLE

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Interaction of central kisspeptin with melanocortin, GABAergic, corticotrophin, and NPY systems on food intake in chickens

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ABSTRACT

Kisspeptin is a key component of reproduction that can directly affect food intake in mammals. There is evidence suggesting that melanocortin, GABA, corticotrophin, and neuropeptide Y (NPY), have a mediatory role in reward; however, how these substances interact with kisspeptin-induced by food intake in birds, remains to be identified. Accordingly, in this study, a total of 10 experiments were carried out to investigate the interplay between kisspeptin and these systems for the control of food intake in neonatal layer-type chickens. In the first experiment, chickens were intracerebroventricular (ICV) injected with saline and Metastin (Kisspeptin, 0.25, 50, and 1 nmol). In the second experiment, saline, Metastin (1 nmol), BIBP-3226 (NPY1 receptor antagonist, 1.25 nmol), and co-injection of Metastin + BIBP-3226 were injected. Experiments 3-10 were similar to experiment 1, except that chickens received BIIE 0246 (NPY2 receptor antagonist, 1.25 nmol), CGP71683A (NPY5 receptor antagonist, 50 µg), Picrotoxin (GABAA receptor antagonist, 1.25 nmol), CGP54626 (GABAB receptor antagonist, 21 µg), astressin-B (CRF1 / CRF2 receptor antagonist, 30 µg), Astressin2-B (CRF2 receptor antagonist, 30 µg), SHU9119 (MC3 / MC4 receptor antagonist, 0.5 nmol), and MCL0020 (MC3 / MC4 receptor antagonist, 0.5 nmol) instead of BIBP-3226. Food intake was subsequently assessed until 120 min after the injection. Based on the findings, Metastin (0.25, 50, and 1 nmol) significantly increased food intake in a dose-dependent manner ($p < 0.05$). However, BIBP-3226 and Picrotoxin inhibited Metastin-induced hyperphagia in neonatal chickens ($p < 0.05$); Whereas, whereas BIIE 0246, CGP71683A, CGP54626, astressin-B, astressin2-B, SHU9119, and MCL0020 had no effect ($p > 0.05$). These results showed that the effect of kisspeptin on food intake might be mediated by NPY1 and GABAA receptors in layer-type chickens.

Keywords

Kisspeptin; GABA, Melatonin; NPY; Corticotropin; Dietary intake; Layer-type chicken

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Number of Pages: 10

Abbreviations

NPY: Neuropeptide Y
ICV: Intracerebroventricular
ARC: Arcuate nucleus: ARC
NTs: Neurotransmitters
Paraventricular nuclei: PVN
Agouti-related protein: AgRP

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Introduction

Appetite control in animals is a complicated process involving both the central nervous system (CNS) and the peripheral nervous system (PNS), modulated by various factors. The hypothalamic regions, particularly the amygdala, nucleus tract, and arcuate nucleus (ARC), can control the appetite via various neurotransmitters (NTs) in CNS [1]. RF amides comprise some peptides with a similar amino acid sequence at the C-terminus, which can play a role in feeding behavior s[2].s Also, it has been documented that RF amides regulate food intake in chickens [3], rodents [4], and humans [5]. Several metabolic activities are provided by Metastin (kisspeptin-10), which is expressed abundantly in the placenta, testis, spine, pancreas, pituitary, and hypothalamic regions [6]. Metastin is expressed in food intake regulated byregulating nuclei, such as ARC and paraventricular nuclei (PVN), and can play a regulatoryan important role in food intake and energy homeostasis [7]. In this regard, Khan et al (2009) reported that ICV administration of Metastin increases food intake in chickens [6].

The ARC functions as a nucleus to control appetite and can facilitate the relationship between appetite-sensing cells in mammals and birds. The appetite is regulated by NPY/agouti-related protein (AgRP), proopiomelanocortin (POMC), and cocaine/amphetamine-regulated transcript (CART) [8]. NPY has six receptors, and NPY1 and NPY5 receptors are responsible for feed intake regulation. However, NPY2 is an autoreceptor that can influence appetite in food-deprived animals. wasPreviously, Yousefvand et al. (2019 and 2020) reported that the ICV administration of the NPY1 and NPY5 receptors antagonists, including B5063 and SML0891, decreased food intake in a dose-dependent manner, while SF22 (an antagonist for NPY2 receptor) increased food intake in broiler chickens [9, 10]. Numerous physiological processes, including cleanliness, temperature homeostasis, training, and energy balancing, are mediated by the melanocortin pathway in the CNS [11]. So far, five melanocortin receptors have been discovered, from MC1R to MC5R; hHowever, the fundamental role of food consumption only includes MC3R and MC4R isoforms [12]. There are many areas of the hypothalamus involved in energy homeostasis and food consumption, such as the ARC, ventromedial hypothalamus (VMH), and PVN, which include the MC3R and MC4R [11, 12, 13].

in their studyPreviously, Bonaventura et al. (2020) showed that ICV administration of agonists and antagonists of the MC3/MC4 receptors reduced and enhanced, food intake, respectively [12]. According to a study conducted by Ahmadi et al. (2019), MC3 and MC4 receptors have a regulatory effect on dietary consumption. The primary process during distress sensitivity is mainly handled by a peptide comprised of 41 amino acids called corticotrophin-releasing factor (CRF). Corticotrophin receptors, especially CRF1 and CRF2, are capable of strong anorectic and thermogenic effects [14]. The CRF1/CRF2 antagonist astressin-B or the CRF2 antagonist astressin2-B abolished icv nesfatin-1's anorexigenic action, whereas an astressin2-B analog, devoid of CRF-receptor binding affinity, did not ICV administrations of adjusted of food in rats [15]. Activation of brain CRF signaling pathways by CRF acting on CRF1 and CRF2 receptors inhibits food intake regulated[16]. Also, Heidarzadeh et al. (2017) showed that astressin-B inhibits hypophagia caused by nesfatin-1 in 3h- food-deprived (FD3) broiler chickens.

In their study Fu et al.(2010) reported that Metastin (0.3, 1, and 3μg/mouse) dose-dependently inhibited the food intake to overnight fasting in mice, and there was an interconnection between Metastin and NPY, and POMC, as Metastin activates POMC, whereas NPY neurons are inhibited [17]. Furthermore, orexigenic melanin-concentrating hormone (MCH) activates neurons inhibited by Kisspeptin [18]. There is a scarcity of data on the effects of Metastin on food intake in chickens because most knowledge on this peptide is sourced from mammalian species.

There is no information about the interplay of metastin with melanocortin, GABAergic, corticotrophin, and NPY systems in the CNS of birds. A novel model to investigate the impacts of a peptide influencing both appetite and adiposity is the use of a line of chickens that vary in these characteristics. The low-weight strain was selected for lower food consumption and raised for egg production, whereas the high-weight strains have higher food consumption which they are famous for meat production [19, 20]. In the poultry industry, studies on layer-type chickens aim to boost the productivity of hens by decreasing food intake and malnutrition. It is critical to understand the potential interactions of kisspeptin with other neurotransmitters in poultry based on comparative physiology. Therefore, this study aimed to investigate the mediatory effects of the central melanocortin, GABAergic, corticotrophin, and NPY systems on kis-

Abbreviations-Cont'd

Proopiomelanocortin: POMC
Ventromedial hypothalamus: VMH
Food-deprived: FD3
Brain-Derived Neurotrophic Factor: BDNF
Gonadotropin-inhibiting hormone: GnIH
Analysis of variance: ANOVABDNF: Brain-Derived Neurotrophic Factor
GnIH: Gonadotropin-inhibiting hormone

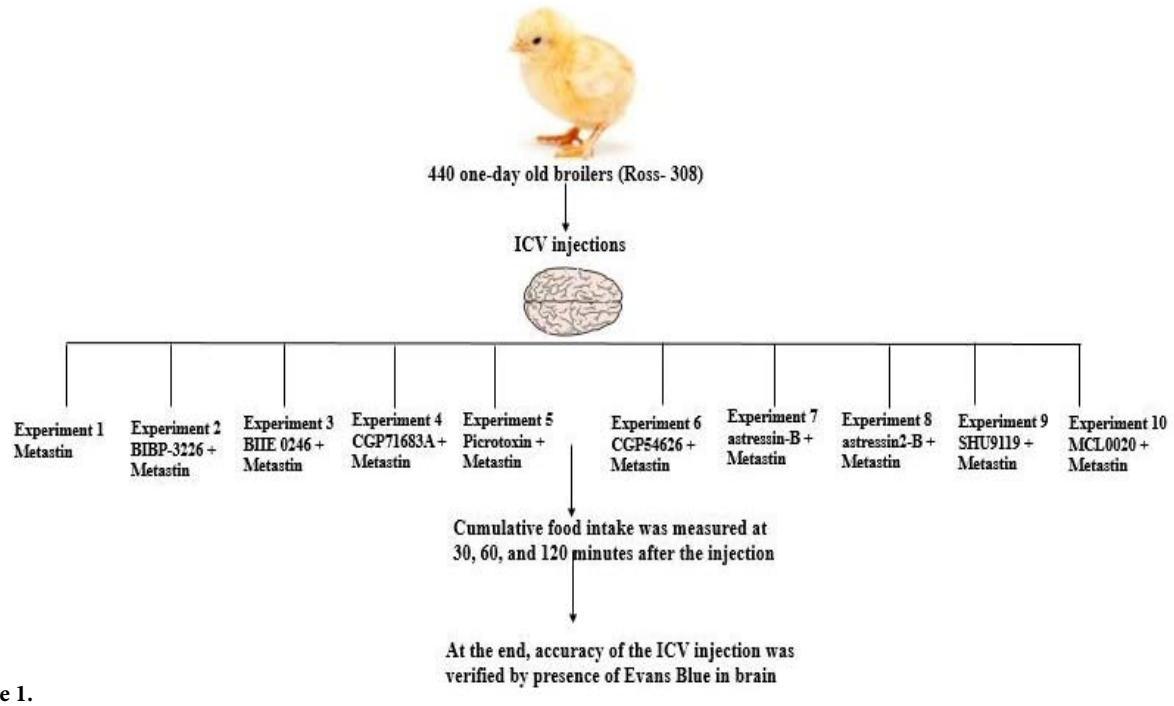

Figure 1.
Flow chart of the experimental procedure.

speptin induced- byfood intake in neonatal layer-type chickens.

Results

In experiment 1, chicks treated with Metastin (Kisspeptin 0.25, 50, and 1 nmol) increased food intake at 30, 60, and 120 min post-injection ($p < 0.05$) (Figures 1 and 2).

In experiment 2, chicks treated with Metastin (1 nmol) increased dietary consumption at 30, 60, and 120 min post-injection ($p < 0.05$). No significant difference was observed between BIBP-3226 (1.25 nmol) and control groups in dietary consumption of chickens ($p > 0.05$). Co-injection of the Metastin + BIBP-3226 inhibited Metastin-induced hyperphagia ($p < 0.05$) (Figure 3).

In experiment 3, Metastin (1 nmol) increased dietary intake at 30, 60, and 120 min post-injection ($p <$

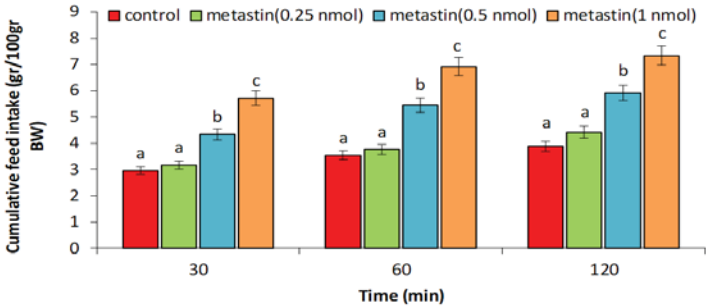

Figure 2.
Effect of ICV injection of metastin (0.25, 0.5, and 1 nmol) on cumulative food intake in neonatal chicken (n = 44). metastin: kisspeptin-10. Data are expressed as mean ± SEM. Different letters (a, b, and c) indicate significant differences between treatments ($p < 0.05$).

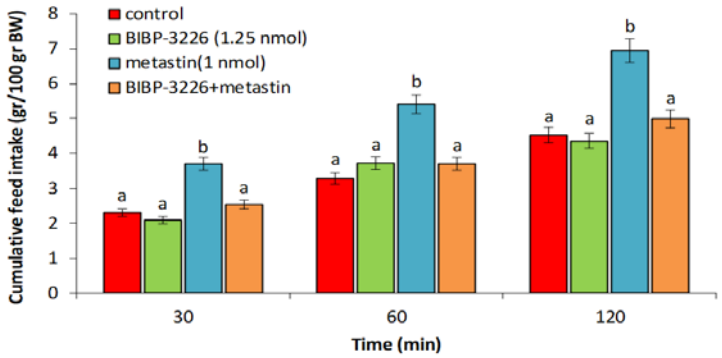

Figure 3.
Effect of ICV injection of BIBP-3226 (1.25 nmol), metastin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n = 44). BIBP-3226: NPY1 receptor antagonist, metastin: kisspeptin-10. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

0.05). Also, no difference was found between the BIIE 0246 and control groups ($p > 0.05$). Metastin + BIIE 0246 co-administration could not alter the hyperphagic effect caused by Metastin ($p > 0.05$) (Figure 4).

In experiment 4, chickens treated with Metastin (1 nmol) increased dietary intake at 120 min after the administration ($p < 0.05$). However, CGP71683A (50 µg) was not correlated with a significant change in food intake at 120 min after the administration ($p > 0.05$). In addition, Metastin + CGP71683A co-administration did not affect Metastin-induced hyperphagia ($p > 0.05$) (Figure 5).

In experiment 5, ICV administration of the Metastin (1 nmol) enhanced food intake at 30, 60, and 120 min post-injection ($p < 0.05$). At 120 min after the administration of Picrotoxin (1.25 nmol), no significant impact on food intake was reported ($p > 0.05$). However, Metastin plus Picrotoxin co-administration attenuated Metastin-induced hyperphagia ($p < 0.05$) (Figure 6).

In experiment 6, co-administration of Metastin plus CGP54626 (21 ng) was not associated with hyperphagic effects caused by Metastin ($p > 0.05$) (Figure 7).

In experiment 7, Metastin (1 nmol) increased food intake in chickens($p < 0.05$). Also, no significant effect on food intake was observed at 120

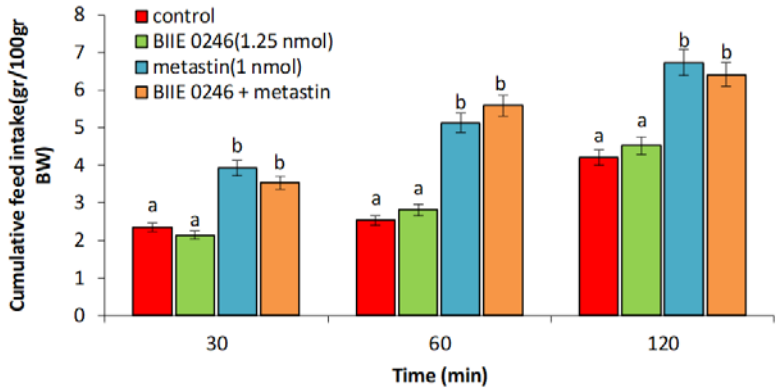

Figure 4.
Effect of ICV injection of BIIE 0246 (1.25 nmol), metastin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n = 44). BIIE 0246: NPY2 receptor antagonist, metastin: kisspeptin-10. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

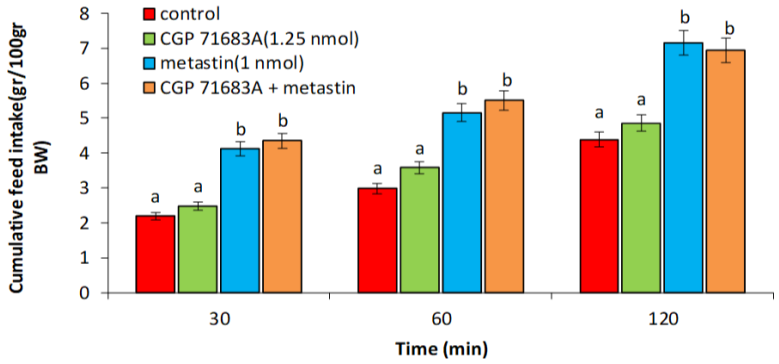

Figure 5.
Effect of ICV injection of CGP71683A (1.25 nmol), metastin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n = 44). CGP71683A: NPY5 receptor antagonist, metastin: kisspeptin-10. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

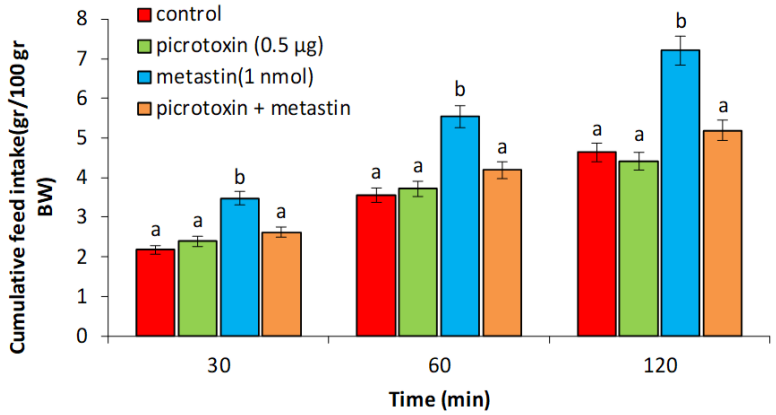

Figure 6.
Effect of ICV injection of picrotoxin (0.5 µg), metastin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n=44). picrotoxin: GABAA receptor antagonist, metastin: kisspeptin-10. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

min following the administration of Astressin-B (30 µg) ($p > 0.05$). No significant impact on food intake was identified at 120 min after the co-administration of Metastin + astressin-B ($p > 0.05$) (Figure 8).

In experiment 8, the injection of Metastin (1 nmol) increased dietary intake ($p < 0.05$). At 120 min after the administration of Astressin-2B (30 µg), no significant impact was observed on dietary consumption ($p > 0.05$). At 120 min following co-administration of Metastin + astressin2-B, no significant effect on food consumption was observed in comparison with Metastin group ($p > 0.05$) (Figure 9).

In experiment 9, ICV administration of the Metastin (1 nmol) increased food intake at 120 min after the injection ($p < 0.05$). However, at 120 min following the administration of SHU9119 (0.5 nmol), no significant impact on food intake was reported ($p > 0.05$). In addition, co-administration of Metastin + SHU9119 could not alter hyperphagic effects caused by Metastin ($p > 0.05$) (Figure 10).

In experiment 10, chicks treated with Metastin (1 nmol) enhanced food intake at 120 min after the injection ($p < 0.05$). At 120 min after administration of MCL0020 (0.5 nmol), no significant change was observed in food intake ($p > 0.05$). Also, co-administration of Metastin + MCL0020 could not alter the hyperphagic effect of Metastin in chickens ($p > 0.05$) (Figure 11).

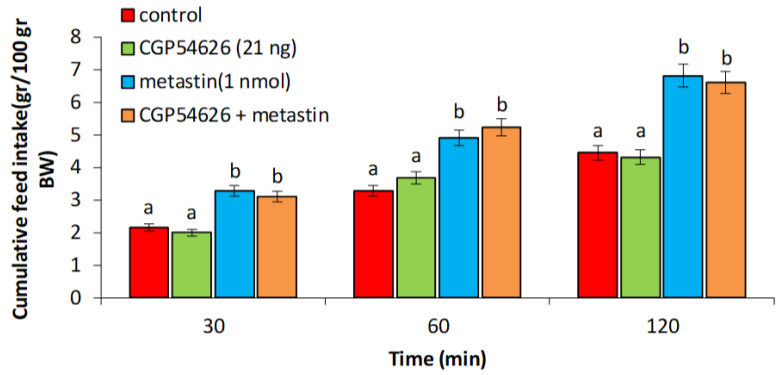

Figure 7.
Effect of ICV injection of CGP54626 (21 ng), metastin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n = 44). CGP54626: GABAB receptor antagonist, metastin: kisspeptin-10. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

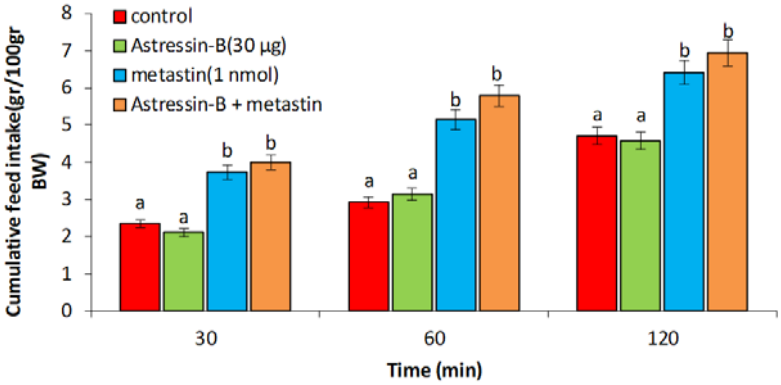

Figure 8.
Effect of ICV injection of astressin-B (30 µg), metastin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n = 44). astressin-B: CRF1/CRF2 receptor antagonist, metastin: kisspeptin-10. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

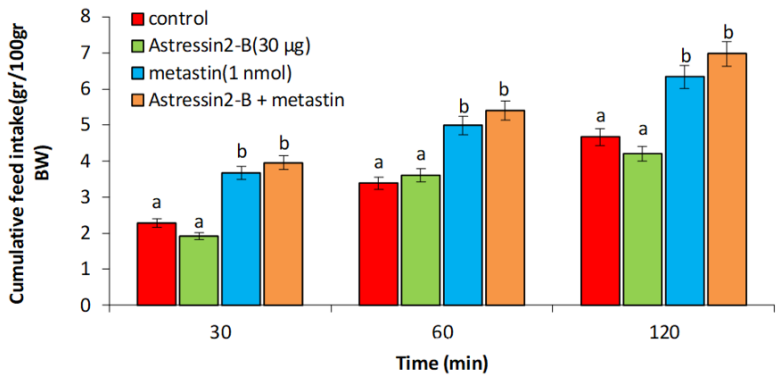

Figure 9.
Effect of ICV injection of astressin2-B (30 µg), metastin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n = 44). Astressin2-B: CRF2 receptor antagonist, metastin: kisspeptin-10. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

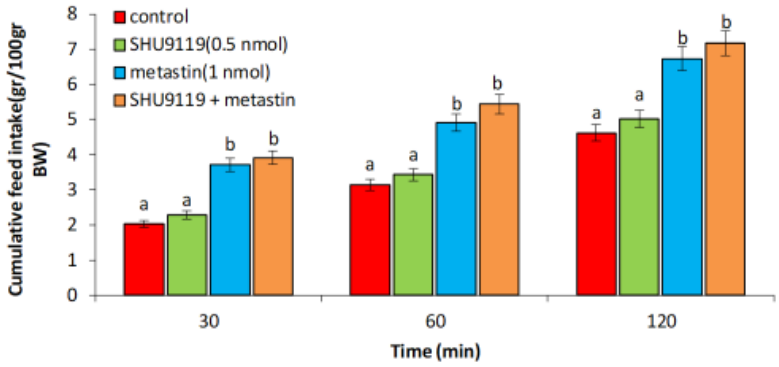

Figure10.
Effect of ICV injection of SHU9119 (0.5 nmol), metastin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n = 44). SHU9119: MC3/MC4 receptor antagonist, metastin: kisspeptin-10. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

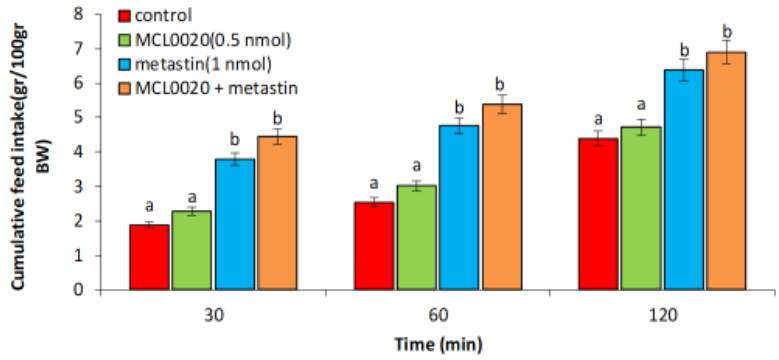

Figure 11.
Effect of ICV injection of MCL0020 (0.5 nmol), metastin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n=44). MCL0020: MC4 receptor antagonist, metastin: kisspeptin-10. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

Discussion

In the present study, we show for the first time that Metastin injected into the lateral brain ventricle at doses (0.25, 50, and 1 nmol) increased food consumption in a dose-dependent manner in neonatal chickens. ICV administration of the Metastin increased dietary consumption in chickens [6]. Stengel et al. (2011) reported that Kisspeptin-10 (0.3, 1, and 3µg/mouse) dose-dependently inhibited the feeding response to an overnight fast by 50%, 95%, and 90% respectively during the 2–3 h period post injection. The 1µg/mouse dose reduced the 4-h cumulative food intake by 28% while intraperitoneal injection (10µg/mouse) did not [7]. Controversial reports exist regarding the role of Kisspeptin as an orexigenic or an anorexigenic factor. For example, One cell research revealed that kisspeptin increases the expression of neuropeptide Y (NPY) which is known to stimulate food intake[27, 29]. In contrast, other authors report that this peptide has an excitatory effect on anorexigenic POMC neurons[17]. Kisspeptin directly excites anorexigenic proopiomelanocortin neurons but inhibits orexigenic neuropeptide Y cells by an indirect synaptic mechanism [17]. Stengel et al. (2011); showed that the centrally injected Kisspeptin reduced food intake by increasing meal intervals in mice[7]. However, central injection of kisspeptin is not able to significantly alter the pattern of food intake, either in rats fed ad libitum or subjected to the previous 12 h of fasting. In good agreement, intracerebral administration of kisspeptin, at a dose effective to maximally elicit LH secretion, failed to change hypothalamic expression levels of NPY, Agouti-related peptide, proopiomelanocortin, and cocaine- and amphetamine-regulated transcript mRNAs (unpublished data) [28]. Perhaps one of the reasons for these discrepancies is the differences between animal species and their physiological differences in regulating centers of food intake.

Co-administration of the Metastin plus NPY1 receptor antagonist inhibited Metastin-induced hyperphagia. Metastin and the NPY2 and NPY5 receptors were not shown to be interconnected during the control of dietary consumption in layer-type birds. Kisspeptin-10 increases the NPY gene expression while inhibiting the Brain-Derived Neurotrophic Factor (BDNF). It is well established that NPY and Kisspeptin neurons could play a critical role in the neural system regulating the pulsatory secretion of GnRH. To understand how NPY regulates Kisspeptin neurons, it has been suggested to be a possible physiological modulator [29]. NPY and Kisspeptin neurons are close in the ARC, and kiSS-1 receptors are expressed in hypothalamic NPY neurons [30]. In addition, NPY null mice showed decreased KiSS-1 mRNA levels at the hypothalamus, whereas exposure to NPY increased the expression of KiSS-1 in hypothalamic N6 cells [31]. Kisspeptin inhibits orexigenic NPY neurons through an indirect mechanism based on enhancing GABA-mediated inhibitory synaptic tone. In

striking contrast, gonadotropin-inhibiting hormone (GnIH and RFRP-3) and NPY, also found in axons abutting POMC cells, inhibit POMC cells and attenuate the kisspeptin excitation by a mechanism based on opening potassium channels [17]. Also, Kisspeptin could directly regulate neuropeptide Y synthesis and secretion via the ERK1/2 and p38 mitogen-activated protein kinase signaling pathways in NPY-secreting hypothalamic neurons [30].

Moreover, central, but not peripheral, injection of kisspeptin-10 was found to decrease food intake in overnight fasted mice [32]. However, kisspeptin neurons are direct targets for regulation by leptin which could act at a post-transcriptional level [33], and it is well known that leptin plays a pivotal role in the hypothalamic regulation of feeding behavior, energy homeostasis, and reproduction [34,35]. Conversely, central injection of kisspeptin-10 was not found to affect feeding in rats [33,36]. Nevertheless, the kisspeptin gene resulted in upregulation in female rats fed on a cafeteria diet, further supporting a discrete role of kisspeptin in energy balance control [37]. Finally, food deprivation or other conditions of negative energy balance, including chronic calorie restriction, led to a significant reduction in hypothalamic kiSS-1 mRNA levels in rats [33,38]. Thus, the opposing findings between animal species might relate to different regulatory mechanisms for food intake among birds and mammals. Given the estimated 300 million years of evolutionary distance between mammals and avians, it is not surprising that significant differences have been found in the activities of several components involved in the regulation of energy homeostasis, such as ghrelin, leptin, and adiponectin[39].

As observed, co-administration of the Metastin plus GABAA receptors antagonist inhibited the hyperphagic effect of the Metastin. However, no interconnection was observed between Metastin and GABAB, CRF1 / CRF2, and MC3 / MC4 receptors on dietary intake regulation among layer-type chickens. It is reported that bicuculline (antagonist for GABAA receptors) could increase Kisspeptin release in the medial basal hypothalamus of prepubertal monkeys [40]. The rhythmic hypothalamic GABA emission variations mean that GABA neurotransmission blockage may be more efficacious in changing peptide secretion in prepubertal than mid-pubertal females [40]. Recently, Ibos et al. (2021) reported that Kisspeptin-8 induces anxiety-like behavior and hypolocomotion by activating the HPA axis and increasing GABA release in the nucleus accumbens in rats [41]. Kisspeptin-10 suppresses the gene expression of the CRF and increases arginine vasopressin and oxytocin in the PVN [41]. However, in the current study, no interconnection was observed between Metastin and CRF1 / CRF2 receptors. As GABAA receptors were selectively activated, gonadotropin discharge was caused by Kisspeptin [42]. So, it is reported that GABA has a regulatory effect on gonadotropic responses to Kisspeptin in male rats [42]. The lateral hypothalamus is a key aspect in feeding and looking for a bonus. Further, the manipulation of GABA transmission in the lateral hypothalamus causes alterations in appetite since stimulation of GABAergic neurons promotes dietary intake, whereas the suppression of these neurons decreases dietary intake [43].

These results showed that the effect of Kisspeptin on food intake is mediated by NPY1 and GABAA receptors in layer-type chickens. The central eating pattern of rats has been the subject of many investigations. It is well established that central appetite control is different in mammals and birds [44]. Therefore, it is concluded that these activities in chickens are governed by the regulatory systems [45]. As observed, no prior study on food intake of the layer chickens was reported concerning Kisspeptin interactions with melanocortin, GABAergic, corticotrophin, and NPY systems. Thus, it was impossible to match our findings with other research. The results of the present study can be used as base information for further studies. Furthermore, it is required to perform merit studies to determine cellular and molecular mechanisms (s) involved in the interaction of Kisspeptin and NPY and GABAergic systems.

Materials & Methods

Animals

In this study, a regional incubator supplied a maximum of 440 one-day layer-type birds (Hy-line) (Morghak Co. Iran). For two days, chickens were stored as groups and randomly moved to isolated enclosures at a temperature of 30 ± 1 °C with 50 ± 2% humidity [21]. Chicks were fed a standard meal comprising 21% crude protein and 2850 kcal/kg metabolizable calories (Chineh Co. Iran). During the research, each chick received an ad libitum diet and fresh water. The birds were FD3; however, they had unrestricted availability to water approximately three hours before ICV administration. At 5 days old, chickens were randomly allocated into ten experiments with four groups (11 chickens per group).

Experimental drugs

Drugs included Metastin (Kisspeptin), BIBP-3226 (antagonist for NPY1 receptors), BIIE 0246 (an antagonist for NPY2 receptors), CGP71683A (antagonist for NPY5 receptors), Picrotoxin (antagonist for GABAA receptors), CGP54626 (antagonist for GABAB receptors), astressin-B (antagonist for CRF1/CRF2 receptors), Astressin2-B (antagonist for CRF2 receptors), SHU9119 (antagonist for MC3 /MC4 receptors), MCL0020 (antagonist for MC3/MC4 receptors) and Evans Blue were purchased from Sigma Co. (Sigma, USA). Medicines were initially dissolved in 100% dimethyl-sulfoxide (DMSO), followed by dilution with 0.85% saline comprised of Evans blue at 1/250 proportion. However, using DMSO in this proportion did not lead to cytotoxicity [22].

ICV administration procedures

To ensure that the average weight in treatment groups was as consistent as conceivable, the chickens were weighed before each administration and then divided into groups according to their body weight. The methods described by Davis et al. (1979) and Furuse et al. (1997) for the application of ICV injections with no anesthesia were performed using a microsyringe (Hamilton, Switzerland). Throughout the right ventricle, a microsyringe pierced 4 mm into the surface of the skull via the tip of the needle. When this technique was used on newborn chickens, it was discovered that no physiological stress was caused by injection [23]. Using an ICV administration with vehicle or medication preparation in a volume of 10 µl, all of the chickens were injected. Finally, the placement accuracy of the injection in the ventricle was verified by the presence of Evans Blue, followed by slicing of the frozen brain tissue [24].

Feeding experiments

In this study, a total of 10 experiments, each with four ICV intervention categories, were designed: 1-4 groups (n=44 in each). In experiment 1, birds received ICV administration of saline and Metastin (Kisspeptin 0.25, 50, and 1 nmol). In experiment 2, chickens were administered with saline, Metastin (1 nmol), BIBP-3226 (1.25 nmol), and co-administration of Metastin + BIBP-3226. In experiment 3, birds received the injection of saline, Metastin (1 nmol), BIIE 0246 (1.25 nmol), and co-administration of Metastin + BIIE 0246. In experiment 4, chickens received ICV injection of saline, Metastin (1 nmol), CGP71683A (50 µg), and co-administration of Metastin + CGP71683A. In experiment 5, chickens received the injection of saline, Metastin (1 nmol), Picrotoxin (1.25 nmol), and co-administration of Metastin + Picrotoxin. In experiment 6, ICV administration of the saline, Metastin (1 nmol), CGP54626 (21 µg), and co-administration of Metastin + CGP54626 was performed. In experiment 7, birds received saline, Metastin (1 nmol), astressin-B (30 micrograms), and co-administration of Metastin + astressin-B. In experiment 8, saline, Metastin (1 nmol), Astressin2-B, and Metastin plus Astressin2-B were administered. In experiment 9, chickens received the injection of saline, Metastin (1 nmol), SHU9119 (0.5 nmol), and co-administration of Metastin + SHU9119. In experiment 10, birds received the injection of saline, Metastin (1 nmol), MCL0020 (0.5 nmol), and co-administration of Metastin + MCL0020. The experimental procedure is shown in Figure 1. Dosage for injections was obtained from previous studies [6, 9, 12, 25, 26]. FD3 chickens were immediately transferred to their respective enclosures and given new water and meal (pre-weighed). Following the administration, the total food consumption (gr) was recorded at 30, 60, and 120 minutes. Consequently, food intake was measured as a percent of the body weight to reduce the impact of weight on the quantity of food consumed. Any chicken was just utilized once for statistical analysis in each treatment group.

The data were expressed as mean ± SEM (standard error of the mean). The two-way repeated measures analysis of variance (ANOVA) using SPSS version 16.0 evaluated the total dietary consumption (as the bodyweight percent) (SPSS, Inc., Chicago, IL, USA). Averages were analyzed employing the Tukey-Kramer test for treatment showing a critical impact using ANOVA. Differences between ICV interventions were statistically significant at $p < 0.05$.

Agreement with ethical criteria

There is no conflict of interest between researchers. This article contains no research conducted by any of the authors with human participants investigations had been carried out in following the Guide for the Care and Use of Laboratory Animals and confirmed by the Committee for Institutional Animal Ethics.

Authors' Contributions

All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Competing Interests

The authors have no conflicts of interest to declare.

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Hydroalcoholic extracts of three *Artemisia* species attenuate dental pulp pain and pain-related abnormal feeding behavior of rats

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ABSTRACT

This study evaluated the therapeutic efficacy of three different *Artemisia* species extracts on capsaicin-induced dental pulp pain and pain-associated changes in feeding behaviors in adult male Wistar rats. The animals were alienated into five groups (n=6), namely sham, capsaicin, and capsaicin groups pre-treated with hydroalcoholic extracts of A. *sieberi*, A. *persica*, and A. *biennis*. Pulpitis was evoked by the intradental administration of capsaicin (100 µg). The plant extracts (200 mg/kg intraperitoneal) were administered 10 min before capsaicin. Pain scores were recorded for 40 min. Afterward, feeding behavior was evaluated within 6 h. All extracts could suppress capsaicin-related dental pulp pain. Furthermore, capsaicin decreased the number of visits to the food and water ports of the feeding behavior evaluation device which led to a reduced amount and duration of meals consumed. These harmful effects of capsaicin on meal duration and frequency were attenuated by A. *persica*. Moreover, the inhibitory effect of capsaicin on food intake and water consumption was suppressed by all the extracts. Overall, the present study showed that *Artemisia* species extracts were useful in suppressing the capsaicin-induced pulpal pain and pain-induced feeding abnormalities.

Keywords

Pulpitis, Capsaicin, Food intake, Artemisia, Rats

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Abbreviations

ANOVA: Analysis of variance
ROS: Reactive oxygen species
CGRP: Calcitonin gene-related peptide
IL: Interleukin
LPS: Lipopolysaccharide
IP: intraperitoneal

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Introduction

Pulpitis is a common primary healthcare problem [1] that rises in response to the stimulation of afferent pulpal nerves by different chemical or mechanical stimuli [2, 3]. In particular, capsaicin has been shown to induce nociceptive behaviors by the activation of polymodal nociceptors. Capsaicin-sensitive fibers constitute the majority of tooth afferent neurons [4]. In addition to the sensory features, the experience of pain correlates with neurophysiological dysfunctions, including altered mood and emotional responses. It may also disrupt metabolic processes [5-7]. In particular, noxious stimuli could alter food intake and food reward/behaviors [8, 9]. There is an inherent and complex relationship between the biochemical mediators, such as histamine, prostaglandins, CGRP, and neuropeptide Y, that contribute to controlling pain and feeding responses [10-13]. Trigeminal nerve dysfunctions have been associated with feeding behavior anomalies in both clinical and preclinical studies [14-16].

Pulpalgia is usually treated with a combination of clinical procedures and chemical medications. However, such treatments have not been sufficient because of various economic, physiological, and psychological difficulties [17, 18]. In this regard, medicinal plants have been frequently used as potential therapeutic compounds for pain originating from the trigeminal nerve [19, 20]. The plant-derived phytochemicals or secondary metabolites, including alkaloids, steroids, tannins, and flavonoids are effective in managing pain and inflammation.

The genus *Artemisia* consists of diverse species that grow in several ecological zones in Asia. In Iran, a number of *Artemisia* species, such as A. *sieberi,* A. *persica*, A. *dracunculus*, and A. *annua*, have been recognized at different altitudes [21]. Artemisia produced analgesia and anti-inflammatory effects. The IP administration of A. sieberi fruits essential oil in mice decreased formalin and carrageenan-induced inflammation [22]. Karimi et al. demonstrated that the methanolic extract of A. *deserti* Krasch could suppress nociception and inflammation in formalin and xylene tests in rats [23]. A. *sieberi* was also shown to be able to inhibit inflammatory and neurogenic pain in mice [24]. In addition, the alleviation of acetic acid-induced writhing pain and thermal nociception has been observed following the oral administration of the essential oil and aqueous extract of absinthium, a species of *Artemisia* [25].

In traditional medicine, *Artemisia* extracts are used to regulate food intake and energy balance [26, 27]. Daily oral treatment with A. annua water extract inhibited adipogenesis in 3T3-L1 adipocytes with no considerable changes in food consumption in a diet-induced obesity mice model [26]. Moreover, A. capillaris extracts could prevent weight gain in obese rats by enhancing lipid metabolism [27]. Whereas, it has been reported that systemic administration of A. absinthium hydroalcoholic extract could not alter food intake and appetite in male rats [28].

It has been indicated that terpenes are the most bioactive chemical compositions of the Artemisia genus [29, 30]. However, there are some differences in the main active compounds among different Artemisia species. The major constituents of A. sieberi have been identified as 1,8-cineole, camphor, α-thujone, p-cymene, terpineol, and camphene [31]. However, the main components in oils obtained from the aerial parts of A. persica Boiss are cis-sabinene hydrate and terpinolene [32]. Furthermore, α- pinene, 1,8-cineole, and camphor have been measured in the oil of A. biennis as the main components [33].

Although previous data have supported the value of Artemisia species against pain and inflammation, their therapeutic value in pulpal pain remains poorly understood maybe due to the unique characteristics of pulpalgia compared to other painful situations. The extracts of Artemisia species contain many bioactive compounds with considerable anti-inflammatory importance. Assessing the efficiency of Artemisia species in dental pulp therapy will help develop cost-effective medicine. The present study used a rat model of capsaicin-induced pulpal pain to assess the effects of the hydroalcoholic extracts of three different Artemisia species, including A. *sieberi*, A. *persica*, and A. *biennis* on pulpalgia. Furthermore, we explored the pain-related changes in the feeding behavior of rats.

Results

Pain assessment

Capsaicin induced significant nociceptive responses compared to the sham-treated group ($p < 0.001$). However, pretreatment of rats with A. sieberi, A. biennis, and A. persica significantly decreased the pain scores at 5, 10, 20, 25, 35, and 40 min intervals after capsaicin administration. In addition, the mean pain scores significantly altered during the 40-min test period among different groups (Chi-Square=21.814, $p = 0.0001$). The post-hoc Mann-Whitney U analysis showed that the nociceptive scores rose in the capsaicin group compared to the sham group ($p =0.02$). However, pretreatment with each of the extracts significantly diminished capsaicin-induced nociceptive responses in rats (Figure 1B).

Food intake

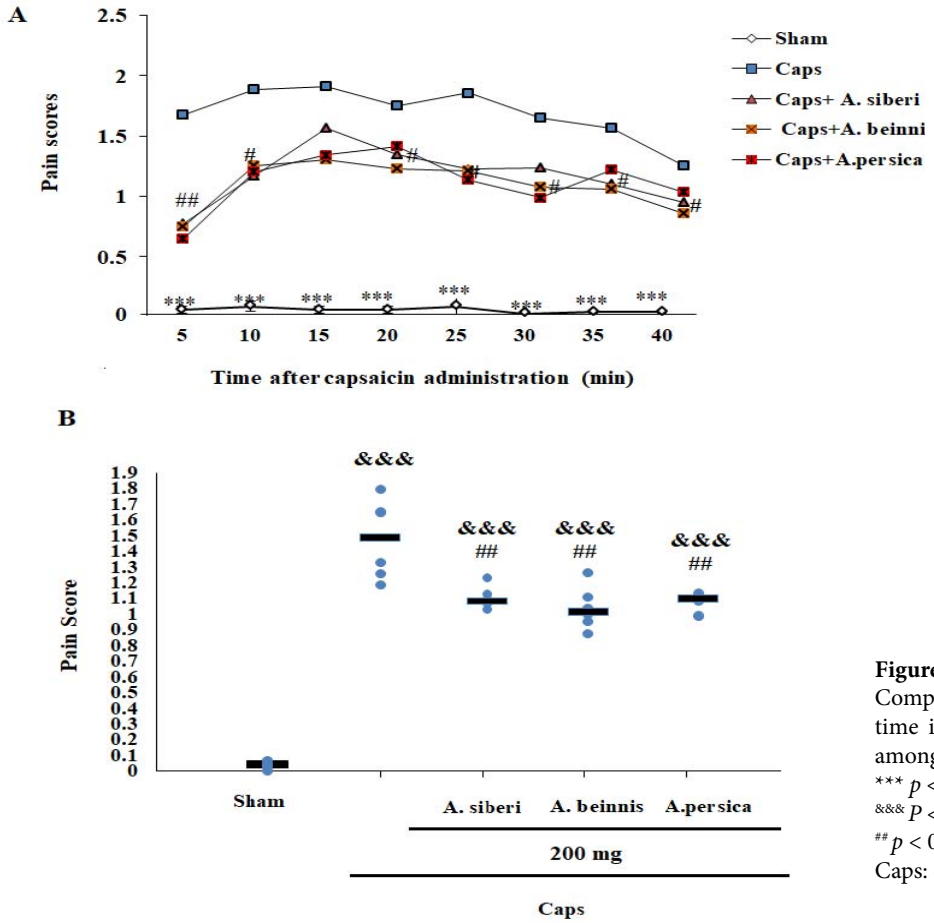

Figure 1.
Comparison of pain scores at different time intervals (A) and total pain scores among experimental groups (B).
*** $p < 0.001$ vs. the other groups;
&&& $P < 0.001$ vs. the sham group;
$p < 0.01$, # $p < 0.05$ vs. the Caps group;
Caps: capsaicin.

The groups showed significant alterations in meal frequency (*Chi*-Square = 20. 266, $p = 0.0001$) and meal duration (*Chi*-Square = 20.839, $p = 0.0001$). As shown in Figure 2, compared to the vehicle group, rats treated with capsaicin presented a decreased number of visits to the meal, along with a shorter time spent there ($p = 0.004$). Capsaicin impact on meal frequency was suppressed by pretreatment with *A. persica* ($p = 0.02$), *A. biennis* ($p = 0.01$), and *A. sieberi* ($p = 0.026$) (Figure 2A). Moreover, meal duration significantly declined in rats treated with *A. persica* ($p = 0.004$), *A. biennis* ($p = 0.01$), and *A. sieberi* ($p = 0.055$) (Figure 2B).

During the 6 h test period, the duration (*Chi*-Square=21.337, $p = 0.0001$) and frequency (*Chi*-Square=22.736, $p = 0.0001$) of water consumption were significantly different between the groups. Capsaicin significantly reduced the duration and frequency of water consumption ($p = 0.004$). In groups of rats treated with capsaicin plus each of the three *Artemisia* extracts, the frequency of water consumption was higher than in the capsaicin group ($p = 0.004$) (Figure 3A). In addition, the time of water consumption, which had decreased due to capsaicin, significantly increased in the groups of rats post-treated with *A. sieberi* ($p = 0.06$), *A. biennis* ($p = 0.02$), and *A. persica* ($p = 0.004$) (Figure 3B).

Significant differences in food intake (*Chi*-Square=17.956, $p = 0.0001$) and water consumption (*Chi*-Square=19.263, $p = 0.0001$) were observed between the groups. As shown in Figure 4, food intake and water consumption rates decreased in rats treated with capsaicin in comparison with the sham group ($p = 0.04$). However, the IP administrations of all three extracts raised food intake in capsaicin-treated rats ($p = 0.03$ and $p = 0.04$) (Figure 4A). Furthermore, water consumption significantly increased in capsaicin plus *A. sieberi* ($p = 0.06$) and *A. persica* ($p = 0.024$) groups in comparison with the animals in the capsaicin group (Figure 4B).

Discussion

Different species of Artemisia have been shown to have analgesic and dietary potentials [22, 26]. In the present study, the IP administration of the hydroalcoholic extracts of A. *sieberi*, A. *persica*, and *A. biennis* decreased capsaicin-induced pulpal pain in rats. In addition, capsaicin application altered the typical pattern of food and water consumption. However, capsaicin-induced reduction in meal duration and frequency were attenuated by pretreatment with *A. persica*. Moreover, all the extracts could improve the reduction in food intake and the time of water con-

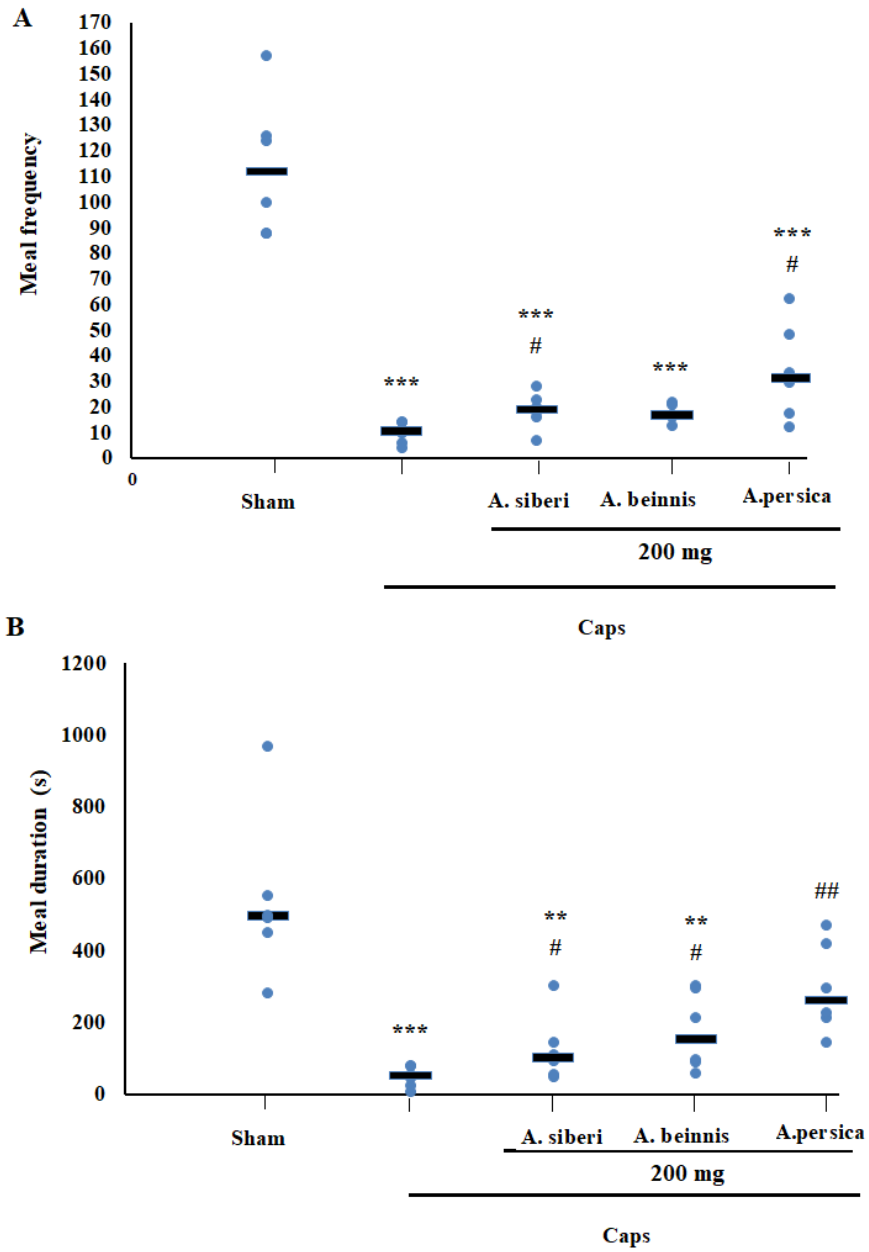

Figure 2.
Comparison of meal frequency (A) and meal duration (B) between capsaicin and capsaicin groups pre-treated with *Artemisia* extracts. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ vs. the sham group; ##$p < 0.01$, #$p < 0.05$ vs. the Caps group; Caps: capsaicin.

sumption caused by capsaicin.

In the present research, all the extracts of tested *Artemisia* species efficiently suppressed capsaicin-induced inflammatory pulpal pain. This study evaluated for the first time the efficiency of the Artemisia genus for the relief of pulpal pain. Therefore, it was not easy to compare our results with findings from previous and similar studies. However, many previous investigations have supported the anti-inflammatory effects of *Artemisia* species. Furthermore, the IP infusion of *A. sieberi* essential oil was able to decrease anti-inflammatory activities comparable to the standard indomethacin in formalin and carrageenan-induced rat paw edema models [22]. Moreover, *A.* persica essential oil significantly diminished the nociceptive behaviors in the formalin and the tail immersion tests in mice [34].

Capsaicin evokes nociceptive behaviors through the tonic activation of polymodal nociceptors in the pulp [4]. It induces proinflammatory mediators in trigeminal afferents which results in pain sensitization and neurogenic inflammation [35]. However, capsaicin-induced nociception and inflammation have been suppressed by anti-inflammatory agents [36]. It has been indicated that *Artemisia* genus extract consists of potent anti-inflammatory compounds, including 1,8-cineole, Limonene, α-Pinene, and α-Terpineol [37]. Therefore, the modulation of capsaicin-induced pulpal pain in the current study may be at least partially attributed to the anti-inflammatory activity of *Artemisia* extracts.

Moreover, pulpal injury stimulates oxidative stress responses by inducing ROS within the impaired cells, resulting in damage to DNA, proteins, and lipids [38, 39]. Elevated ROS levels negatively affect antioxidant enzyme activity and induce cellular toxicity. Mean-

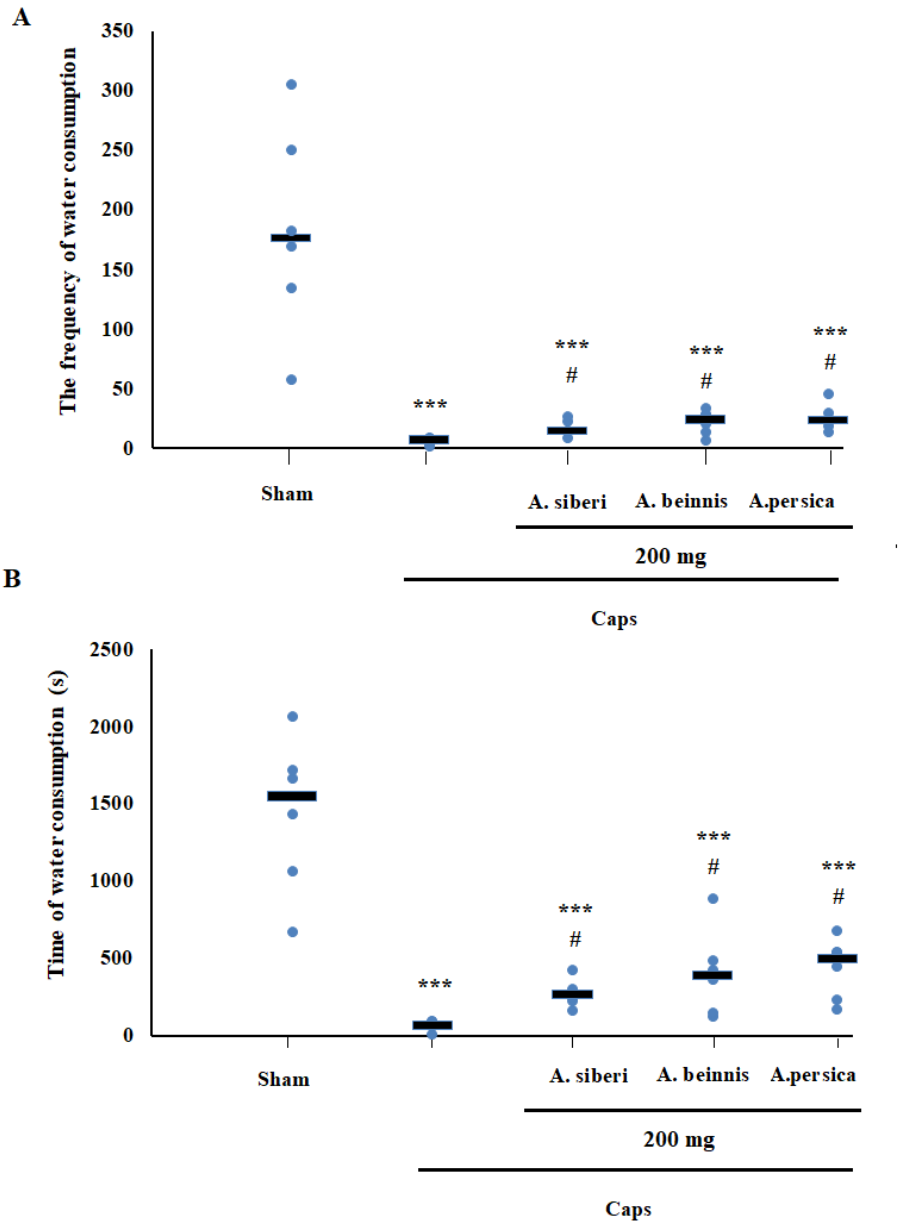

Figure 3.
Comparison of the frequency (A) and duration (B) of water consumption between capsaicin and capsaicin groups pre-treated with *Artemisia* extracts.
***$p < 0.001$ vs. the sham group; #$p < 0.05$ vs. the Caps group; Caps: capsaicin.

while, natural products with potential ROS scavengers are recognized to suppress tissue damage in pulpitis [40, 41]. In this regard, Artemisia species are well characterized as a source of natural antioxidants. It has been indicated that pre-treatment with the extracts of the aerial parts of *A. biennis* could increase the activity of superoxide dismutase and mitochondrial membrane potential, and suppress intracellular levels of ROS in the PC12 cells [42]. In addition, the in vitro administration of A. annua extract has been shown to reduce the levels of oxidative enzymes, malondialdehyde, and 8-OH-dG. On the other hand, it increases the activity of the antioxidant enzyme NQO1 in D-galactose-treated mice [43]. Moreover, *A. vulgaris* extract led to lower nitric oxide scavenging activity, enhanced levels of blood glutathione, and higher superoxide dismutase activity in rats [44]. Therefore, in the current study, the potential antioxidant activity of *Artemisia* extracts might contribute to capsaicin-induced pulpal pain attenuation.

Based on the data, capsaicin-induced pulpalgia was associated with food and water intake abnormalities in rats. In support, previous studies raised a similar argument related to the destructive effects of pain on normal feeding patterns in rodents. It has been indicated that temporomandibular joint pain reduces food intake in rats [45]. Martin et al. reported that upper abdominal surgery disrupts feeding behavior in rats [46]. There are complex associations between brain regions and neurochemical substances involved in controlling feeding and pain processing. Pain experience has been found to disrupt the balance of neurochemicals involved in feeding behaviors [47]. Interestingly, sucrose increased neural

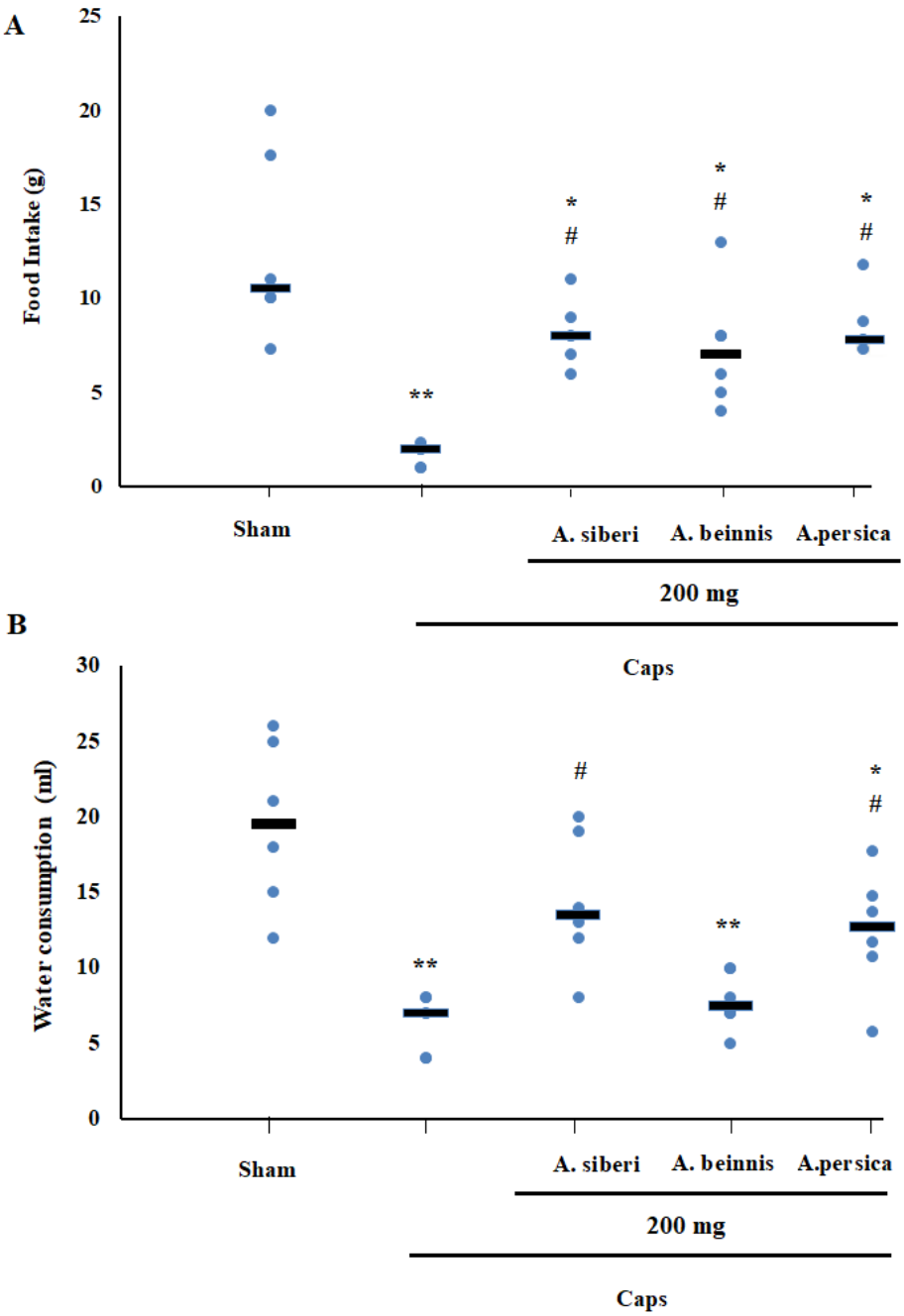

Figure 4.
Comparison of food intake (A) and water consumption (B) between capsaicin and capsaicin groups pre-treated with *Artemisia* extracts.
$^{**}p < 0.01$, $^{*}p < 0.05$ vs. the sham group; $\#p < 0.05$ vs. the Caps group; Caps: capsaicin.

activity within the nucleus raphe magnus and periaqueductal grey matter as critical supraspinal pain modulation regions [48].

Here, abnormalities in food and water intake associated with dental pulp pain were suppressed in rats treated with *Artemisia* extracts. Previous studies have also suggested an important role for the Artemisia genus in regulating metabolic processes and energy expenditure [27]. In addition, daily oral administration of *A. annua* increased lipid peroxidation in a diet-induced obesity mice model [26].

It has been indicated that anti-inflammatory compounds can regulate feeding behaviors. Central administration of IL-10 decreased the peripheral LPS-induced diminished food consumption in rats [49]. Furthermore, the intra-ventromedial hypothalamus infusion of an IL-1 receptor antagonist could disrupt food intake in tumor-bearing anorexic rats [50]. Moreover, the systemic administration of CGRP reduced food consumption and plasma metabolic hormones in rats [51]. As a result, the ability of *Artemisia* species extracts to decrease pain-evoked abnormal feeding behavior may be due to anti-inflammatory compositions, such as sesquiterpenes, scoparone, and flavonols.

In this study, *Artemisia* extracts augmented water intake in capsaicin-treated rats, which may be secondary to increased food consumption. Moreover, this effect may be somewhat related to high locomotor activity. Overall, our results provided a shred of evidence for the efficiency of A. *sieberi*, A. *persica*, and

A. biennis extracts in reducing dental pulp pain and pain-induced food intake anomaly in rats. It indicates an impending value of *Artemisia* extracts in the treatment of dental pain and eating disruption in pulpal inflammation.

Materials & Methods

Animals

This study was conducted in adult male Wistar rats (230-250 g). The animals were maintained in a room with a constant temperature (22 °C ± 1 °C) under a 12:12 h light-dark cycle. There was ad libitum access to food and water. All the procedures were certified by the relevant Ethical Committee of Shahid Bahonar University of Kerman, Kerman, Iran (98.9).

Nociceptive induction

The rats were anesthetized with a low concentration of carbon dioxide. A cavity (2 mm^3) was arranged in the left mandibular incisor using a small fissure bur in a high-speed handpiece. The hole was restored with a small cotton pellet saturated with capsaicin solution. Next, the animals were individually located in a box (30 cm^3) with a mirror set at a 45° angle under the floor to show the responses of the rats. Pain scores were assessed as follows: score 0: normal grooming of body and facial behavior, score 1: mild shaking of the inferior jaw, score 2: continuous grooming of injected zone with the forelimbs, and score 3: extensive rubbing of the mouth. The nociceptive behavior was evaluated in a 40-min test. The nociception scores were calculated using the following formula:

$$\text{Pain score} = \frac{\text{score 0(s)* 0+ score 1(s)* 1+ score 2(s)*2+ score 3(s)*3}}{300}$$

Plant material

Fresh leaves and twigs of *A. persica* and *A. biennis* were collected from the Hezar mountains (Kuh-e Hazaran), and *A. siberia* was collected from the Bidkhoon area, Kerman province, central part of Iran. The samples were evaluated by Dr. Mansour Mirtadzadini. The coupon varieties were placed at the Herbarium of Shahid Bahonar University of Kerman, with the Herbarium codes 3386, 3385, and 3384 for *A. persica, A. biennis,* and *A. Siberia,* respectively. The leaves and twigs were dried at room temperature, cut into small pieces, and distilled. The powder form of *A. persica, A. biennis,* and *A. siberia* (each 50 g) was extracted with methanol (200 ml) three times at room temperature. The methanol extracts were mixed and vaporized by a vacuum rotary evaporator at 45 °C to the dried compound form (yield 2.6% w/w). The provided extracts were then lyophilized and kept in the dark desiccators at +4 °C until tested. They were powdered and extracted with methanol. Afterwards, the solvent was separated, and the extract was determined on a water bath to achieve a dry deposit.

Experimental procedures

The study groups, each with six subjects, included the sham group which received IP normal saline and intradermal capsaicin vehicle, the capsaicin group which received intradermal capsaicin (100 µg), and three groups that received the IP injections of *A. persica, A. sieberi,* and *A. biennis* extracts at a concentration of 200 mg/kg 10 min before capsaicin administration. To evaluate the food intake behavior of animals, 1 h after capsaicin administration and nociceptive assessment, the rats were placed in the middle of an open field-like sealed black plexiglas box (60 cm × 60 cm × 30 cm). A load sensor was mounted underneath the apparatus for recording the behavior of rats, and software processed the location, as well as food and water consumption of animals. There was a port in one of the interval walls that was opened at the middle square allowing access to food and water. The frequency and duration of consuming the meals and the time spent near the food and water port were recorded for 6 h.

Statistical analysis

Statistical analysis was performed using the SPSS software (IBM, USA). The data are summarized as median and range. The statistical differences between the study groups were analyzed by the non-parametric Kruskal-Wallis H test. Moreover, the Mann-Whitney U test was used to compare differences between two independent groups. p-value < 0.05 was considered statistically significant.

Authors' Contributions

J.H. designed the experiments. F.H., A.S., and M.K.H. performed the experiments. R.K. supervised the study and analyzed the data. M.R. and M.A. drafted the manuscript. All authors have read and endorsed the final draft of the manuscript.

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Competing Interests

No competing interests to declare.

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Serological diagnosis and risk factors associated with bovine paratuberculosis in the municipality of Tuta, Colombia

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ABSTRACT

Bovine paratuberculosis or Johne's disease is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), which affects domestic and wild ruminants around the world. The clinical presentation of MAP infection is characterized by chronic diarrhea unresponsive to treatment. The objective of the present study was to investigate the seroprevalence and risk factors associated with bovine paratuberculosis in cattle herds of Tuta, Boyacá, Colombia. This descriptive cross-sectional study with simple random sampling was performed on 882 blood samples taken from cattle of different racial and age groups. Blood samples were processed using an indirect enzyme-linked immunosorbent assay (PARACHEK® 2 Kit, Prionics AG, Switzerland). The obtained data were analyzed by the statistical software Epi Info. In this study, a general seroprevalence of 3.1% was found, and seropositivity in females was 3.6%. The highest prevalence of antibodies against MAP was in individuals > 4 years (5%) and the Jersey breed (4.8%). Therefore, the age of over 4 years was identified as a risk factor associated with MAP. Moreover, a statistical association was found between management and biosafety variables, such as pen management (p = 0.012), feeding with concentrate (p = 0.012), and the presence of diarrhea on the farm (p = 0.048). It could be concluded that the disease is present in Tuta, however, considering factors, such as the chronicity of the disease and the diagnostic method used, it is expected that the number of infected animals is much higher than presented in this research.

Keywords

Paratuberculosis, Mycobacterium avium subsp. paratuberculosis, indirect ELISA, seroprevalence, risk Factors, DeCS

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Abbreviations

MAP: *Mycobacterium avium* subsp. *paratuberculosis*
PTB: Paratuberculosis
DeCS: Descriptores en Ciencias de la Salud (Descriptors in Health Sciences)
ELISA: Enzyme-linked Immunosorbent Assay
PPV: Positive Predictive Value
NPV: Negative Predictive Value

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Introduction

Johne's disease or PTB is an intestinal infectious inflammatory disorder associated mainly with domestic and wild ruminants, which affects both animals and producers due to decreased milk production, premature slaughter, and reduced weight gain in animals infected with MAP [1].

PTB presents complex epidemiology and it has been reported that animals are infected at an early age but do not develop clinical signs until several years after the initial infection [2]. The clinical expression of MAP infection is characterized by chronic diarrhea unresponsive to treatment, leading to emaciation and ultimately, sacrifice or death [3]. PTB causes inflammation and the malfunction of the intestinal tract, with gross pathology showing thickened and edematous intestinal walls. In turn, these intestinal injuries affect the absorption of nutrients and proteins, causing muscle wasting and lower productivity [4].

In subclinical infection, adult carriers show no specific clinical signs, while may be affected by other abnormalities, such as mastitis or infertility. Milk production decreases, but vital signs are within normal limits. In advanced clinical disease, emaciation is the most obvious abnormality and is often accompanied by intermandibular edema, which tends to disappear as diarrhea develops [5].

However, PTB has not been demonstrated to be a zoonosis. Although the causative agent of the disease has occasionally been detected in some patients with Crohn's disease, its role as a human pathogen has not been fully accepted [4]. Annual losses of $198 million, $75 million, $56 million, $54 million, and $17-28 million have been reported in the United States, Germany, France, New Zealand, and Canada Due to the disease, respectively [6].

Diagnostic tests for Johne's disease are improving, but the accurate detection of all infected animals, especially those at an early stage of infection and transmitting the organism within a herd, is not yet possible. This fact makes the test and discarding strategies ineffective [7] precisely among the indirect tests, ELISA is used more frequently and is indicated mainly for seroprevalence analysis in countries that adopt surveillance programs. The advantages of this technique include wide availability, low cost, and fast results [8].

Although PTB has been known for decades, research in Colombia has been insufficient to accurately reflect the epidemiological situation, economic impact, and public health impact of the disease on the country. This limits the knowledge about the magnitude of MAP circulation in animals, humans, the environment, and food in the Colombian territory [9]. Therefore, it is necessary to conduct further studies to establish the seroprevalence and risk factors of MAP. This type of study allows knowing more about the disease behavior in the country, especially in a municipality, such as Tuta, whose economy is based on the production of bovine milk, and where the effect of this disease is not known.

In addition, these studies in the future serve to generate sufficient information for both governments and farmers, which will allow them to make sound decisions on the control and management of PTB. Currently, PTB control and management continue to be an obligation for the owner of the farms. Although this is a notifiable disease according to the World Organization for Animal Health (OIE) [10], there is very little government effort for detection and control. As mentioned before, both producers and veterinarians only get to see the repercussion of the disease in the terminal stages or at necropsy after unsuccessfully trying to treat the animals with medications, such as anthelmintics and antibiotics.

Results

In our study, an apparent seroprevalence of 3.1% (27/882) and a true prevalence of 4.4% were found, with a PPV of 100% and an NPV of 98.6%. Females were the only seropositive with 3.6% (true prevalence: 5.1%, PPV 100% and NPV 98.4%). Animals > 4 years (5%) and the Jersey breed (4.8%) had a higher prevalence of antibodies against MAP than other groups. On the other hand, no cattle in the age group of 2-4 years and the Aryshire and Cebú breeds were seropositive to the disease (Table 1).

In the present study, disease presentation had a significant statistical association with the ages 2-4 and > 4 years, cattle gender, presence of pens in the herds, concentrate supply, and diarrhea presentation in animals ($p \leq 0.05$). Moreover, it was established that the Ayrshire breed and age < 1 year were protective factors for the presentation of PTB in the evaluated cattle, while the supply of concentrate and age > 4 years were determined as possible risk factors for the presentation of PTB (Tables 2 and 3). The logistic regression model revealed that the age older than 4 years was a risk factor for the presentation of bovine PTB in the evaluated herds (Table 4).

Discussion

The overall seroprevalence in our study was 3.1%, which is lower than the report of the Department of Boyacá by Bulla-Castañeda et al. (2020) where a prevalence of 10.9% was found [11]. Similarly, it differs from the national results in beef cattle in the

Table 1.
Apparent prevalence (AP) and real prevalence (RP) of bovine paratuberculosis by breed and age group in cattle from Tuta, Boyacá.

Category	number	Positives	AP %	RP %	PV + (%)	PV- (%)
Age Group						
< 1 year	171	2	1.2	1.7	100	99.5
1-2 years	204	5	2.5	3.6	100	98.9
2-4 years	107	0	0.0	-	-	-
> 4 years	400	20	5.0	7.1	100	97.7
Breed						
Holstein	498	19	3.8	5.4	100	98.3
Ayrshire	10	0	0.0	-	-	-
Jersey	21	1	4.8	6.9	100	97.8
Normando	250	5	2.0	2.9	100	99.1
Cebú	22	0	0.0	-	-	-
Cruces	81	2	2.5	3.6	100	98.9

PV +: Positive predictive value
PV -: Negative predictive value

Table 2.
Possible risk factors associated with bovine paratuberculosis infections.

Variable	Category	PR[1]	CI 95%[1]	*p*-value
Breed	*Holstein*	*1.018*	*0.9951-1.0415*	*0.09833374*
	Normando	*0.9849*	*0.9624-1.0079*	*0.17619706*
	Ayrshire	0.969	0.9576-0.9806	0.73159449
	Cebú	0.9686	0.957-1.0803	0.50037238
	Cruce	0.9933	0.9574-1.0306	0.54151327
	Jersey	1.0183	0.9247-1.1213	0.48342852
Age	< 1 year	0.9763	0.9555-0.9975	0.07933193
	1-2 years	0.9919	0.9666-1.0177	0.37884669
	2-4 years	0.9652	0.9523-1.0782	0.02878119
	>4 years	1.0373	1.0118-1.0636	0.00206385
Sex	-	0.964	0.9508-1.0775	0.01212846

* Significance is denoted by a *p*-value <0.05.
[1] The results are shown as prevalence ratio (PR) and 95% confidence interval (95% CI).

Department of Antioquia [12], in which the presence of bovine PTB was 33.8% and 17% in dairy biotype breeds from the same department [13]. The difference in the reported results mainly results from the response of the ELISA technique to MAP due to the stage of infection. Subclinical cases are usually seronegative, while animals with a high bacterial load are seropositive. Therefore, in the early stages of infection in most female cattle, when fecal excretion is low, the humoral antibody response is below the detection limit of serological tests [14, 15].

Comparing the results of this study with other investigations in Africa, Europe, and Latin America reveals that the values obtained in the present research are similar to those reported by various authors. Elmagzoub et al. (2020) and Ozsvari et al. (2020) observed a seroprevalence of 5.5% in dairy herds in Hungary, while a seroprevalence of 6.3% was found in Sudan [16, 17]. In Latin America, a seroprevalence of 2.1% was reported in dairy cattle and 9% in beef cattle in Argentina, and the first value was close to what was found in Tuta [18]. On the other hand, seropositivity values ranging from 6.3% to 10.7% have been established in Brazil, Chile, and Argentina [19–21].

Regarding the gender of the specimen, in our study, there was a significant statistical association between females and disease presentation, which is consistent with what was established by Doria-Ramos et al. (2020) who indicated that female cows were affected with PTB more than males (OR = 4.37) [22]. According to Hole & Maclay (1959), there is a certain degree of susceptibility in certain bovine genetic lines. Furthermore, various reproductive states of females, such as childbirth and lactation, generate immunological alterations that can make females more prone to acquiring the infection [23–26].

In the present study, the highest seroprevalences were determined in Jersey (4.8%) and Holstein (3.8%) breeds, which coincided with the significant differences in positivity in different dairy breeds. Females of the

Table 3.
Possible risk factors associated with bovine paratuberculosis infections, according to the management and biosafety variables of the farms.

Variable	Category	PR[1]	CI 95%[1]	p-value
Management of animals in pen	-	0.964	0.9508-1.0775	0.01212846
Natural service	-	1.0283	1.0069-1.0502	0.01673262
Artificial insemination	-	1.0088	0.9856-1.0325	0.30021614
Presence of other species in the farm	-	1.007	0.9836-1.0309	0.36282154
Own animals grazing in leased land	-	0.033	1.0204-1.0457	0.31903584
No presence of fence / inadequate or damaged fences	-	0.9961	0.972-1.0209	0.47205467
Hand milking	-	1.0126	0.9874-1.0384	0.20646591
Mechanical milking	-	0.9909	0.9674-1.0149	0.2846629
Silo feeding	-	1.0235	0.996-1.0518	0.05268572
Hay feeding	-	1.006	0.9723-1.0408	0.43831931
Feed with concentrate	-	1.0383	0.9793-1.1009	0.08539856
Quarantine upon entry of new animals	-	0.9833	0.9498-1.0181	0.1970303347
Presence of diarrhea	-	1.0240	0.9975-1.0513	0.0485341893
Herd size	Small herd (< 10 animals)	1.0106	0.9773-1.0451	0.3153541107
	Large herd (> 10 animals)	0.8873	0.8193-1.1031	0.1779873347

* Significance is denoted by a *p*-value <0.05.
[1] The results are shown as prevalence ratio (PR) and 95% confidence interval (95% CI).

Table 4.
Analysis of the variables as possible factors of paratuberculosis in cattle from Tuta, Boyacá.

Variable	Odds Ratio (OR)	Lower confidence interval (LCI 95%)	Upper confidence interval (UCI 95%)	*p*-value *
Concentrate	*5.8932*	0.7631	45.51	0.089
> 4 years	3.5707	1.4942	8.5329	0.0042

* Significance is denoted by a *p*-value <0.05.

Channel Island breeds (Jersey and Guernsey) were more likely to be seropositive for MAP than the females of other breeds [27, 28]. Genome-wide association analysis in Jersey cattle by Kiser et al. (2017) concluded that some variables contribute to the diversification in the reported heritability estimates, including the level of exposure to MAP in a given herd, precision of diagnostic tests and the samples used, handling of the specimens, and sample size [29]. In addition, a genetic predisposition of certain bovine breeds to MAP infection has been reported [1, 30, 31].

We found that the age of > 4 years was a risk factor for PTB presentation, which might be related to the nature of the disease because the incubation period of the disease is 5 years [32]. According to Fecteau (2018), cattle develop resistance with age and are usually infected when they are calves. Consequently, in a few animals, the infection evolves before 2 years of age, but from 2 to 6 years of age [1]. A large proportion of infected animals seem to develop anti-inflammatory immune responses characterized by IgG antibodies, which would explain why in this study the older individuals were the ones with the highest seroprevalence [33].

Another important factor to take into account for

the diagnosis of PTB is the test used for its identification. The capacity of a diagnostic test is determined by the age of an animal due to the chronic nature of MAP infection [34, 35]. Sweeney et al. (2016) reported a sensitivity of 15% for disease detection by ELISA in young subclinical cows and a sensitivity of 87% for detecting clinical cases that normally occur in older than 2 years by the same ELISA [36]. Therefore, the high seropositivity by ELISA in animals older than 4 years of age and its consideration as a risk factor for PTB in our study is in line with the reports of other investigations [20, 22, 37–41].

Regarding the management and biosecurity measures, our results showed that the presence of diarrhea had statistically significant associations with the variable feeding with concentrate and pen management. Eisenberg et al. (2012) reported significantly less frequent detection of MAP outside the stable compared to inside the stable because the high dilution of airborne contamination contained MAP mainly inside the barn, which would explain the association of the mentioned variables [42]. If adequate sanitary measures are not followed, for example, the MAP-contaminated manure is carried on the shoes when passing from the pen of adult cows to the pen of young calves, the disease spreads more easily. Therefore, some authors recommend separating newborns from adults, even when there is enough space between the pens [43]. In addition, the dust that is continuously produced in the animal housing by the movement of animals is made up of skin, hair, dry fecal matter, as well as feed and bedding material that can be spread throughout the barn by air and carry MAP particles [44].

Storing concentrate in places close to the management area of the adult cattle without the frequent cleaning of these sites, makes the feed contaminated more easily, As a result, the management of the concentrate in farms is considered important for the prevention of PTB. On the other hand, there is research that reports an association between feeding with concentrate and seropositivity for the disease [11]. Therefore, it is necessary to disinfect the storage area and leave the barn empty for two weeks to effectively reduce the presence of MAP on surfaces and in the environment as mentioned by Eisenberg et al. (2011) [42].

We also found a statistical association between the positive animals and those who presented diarrhea, which is very important because diarrhea is considered a clinical sign of the disease. The latter association has already been revealed in some studies. For example, in China, cows from two dairy farms in Tai'an City, Shandong Province, that exhibited this clinical sign and were resistant to antibiotic treatment were found to be positive for PTB based on clinical inspection and histopathological examination [45]. In Turkey, in a study on animals with chronic diarrhea, the disease was detected more in animals with this clinical sign [46].

In the current research, the size of the herd did not have an association with the disease. On the other hand, some investigations observed changes in disease behavior depending on the size of the herd, showing that an increase in the number of animals rises the chance of having positive subjects [47, 48]. However, further research is required, especially because the average size of dairy herds in the area continues to increase [49]. Currently, the size of the herds is not large enough to make a statistically significant difference.

The presence of the disease in the municipality of Tuta is confirmed. However, the seroprevalence was not high due to the chronic course of the infection. In cattle > 4 years the seroprevalence was significant and was considered a risk factor. Therefore, it is presumed that the number of infected animals is much higher than that presented in this work considering the number of subclinically infected animals that do not show a strong immune response diagnosed as seropositive.

Feeding with concentrate presented a statistical association as well as management in the pen, showing the importance of taking different sanitary measures that will notably influence disease control. Moreover, a statistical association was identified with diarrhea, which could be taken as a biosecurity measure on farms because animals with this clinical sign can be separated and prevent the spread of the disease.

Further studies using different diagnostic methods in the area that specify the impact of the disease on the cattle herds of the municipality of Tuta are recommended. These investigations may serve as a basis for an adequate control plan and prevention of bovine PTB.

Materials & Methods

Geographic location

Tuta is located 26 km from the city of Tunja on the Briceño-Sogamoso double carriageway. It is part of the central province of the Department of Boyacá and is located in the green valleys of the Tuta River, which is part of Alto Chicamocha. The urban area is located at north latitude 05º 41' 36" and west longitude 73º 13' 51" at 2600 meters above sea level with an average temperature of 14 °C [50]. The relative humidity throughout the year is 75%. In the municipality of Tuta, there are two rainy periods during the year, the first of which is between April and June and the second rainy period is between September and November [51].

Sample size

According to the Livestock Census of the Instituto Colombiano Agropecuario (Colombian Agricultural Institute) (ICA), carried out during the two annual vaccination cycles against foot-and-mouth disease and brucellosis, there are 26,411 cattle in the municipality of Tuta [52].

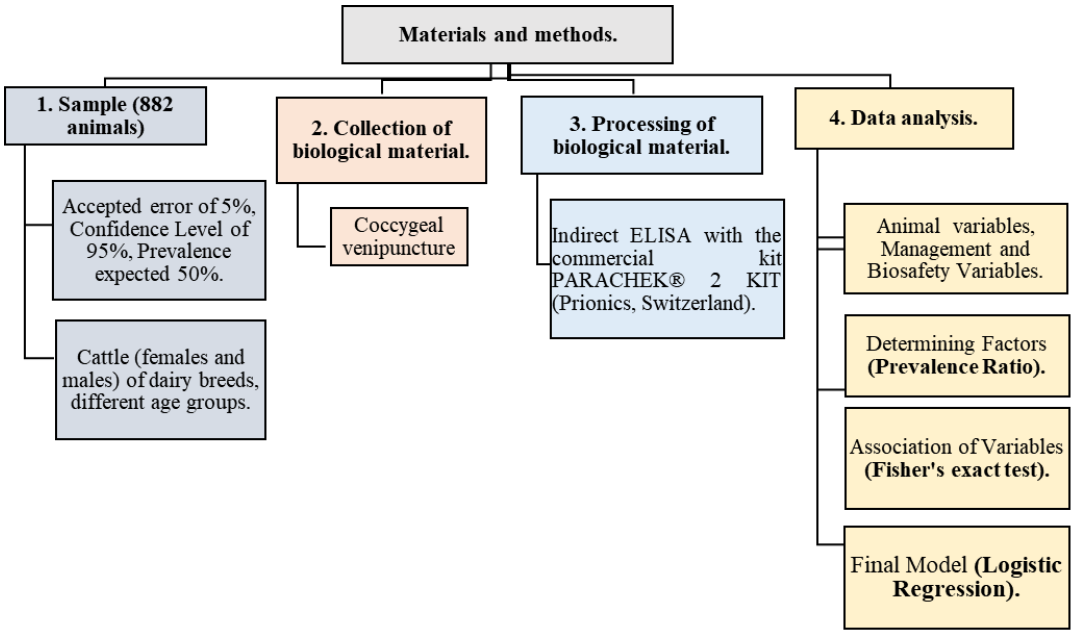

Figure 1.
Summary of the instruments used and description of the steps taken in this research

A sample size of 882 animals with a sampling fraction of 3.44% was determined by the following formula:

$$= \left(\frac{Z\alpha_{/2\sqrt{p(1-p)}}}{E} \right) = \frac{Z^2\alpha/2 \cdot p(1-p)}{E^2}$$

Where n is the sample size, Zα represents constant at the 95% confidence interval, p denotes expected prevalence, and E is the acceptable margin of error. An accepted error of 5%, a confidence level of 95%, and an expected prevalence of 50% were considered because no studies of this type have been carried out in the municipality.

Collection of biological material

Blood samples were collected from the female and male cattle of dairy breeds in different age groups. The blood specimens were obtained by coccygeal venipuncture using needles of 16 g × 3, 18 g × 3, and 16 g × 1 gauge. Before taking the blood samples, the area was depilated and disinfected with alcohol to facilitate taking the sample and avoid contamination. For blood collection, the vacuum tube system (Vacutainer®) was used, which provides an aseptic condition and preserves the samples due to being a closed system [53].

Processing blood samples

The samples were centrifuged at 2500 rpm for 10 min to separate the cells from serum. Subsequently, using a Pasteur pipette, the serum or supernatant was separated and transferred to a storage tube to perform the test [53]. Serum samples were assessed using indirect ELISA by commercial PARACHEK® 2 KIT (Prionics, Switzerland) (sensitivity 70% and specificity 100%) following the manufacturer's instructions. Those samples whose percentage of positivity was ≥ 15% of the cut-off point were determined as positive.

Variables

The evaluated variables were classified into two categories of animals and management. The management and biosafety variables included management in the pen, the use of artificial insemination or natural service, presence of other species on the farm, grazing on rented land, fence general condition, manual or mechanical milking, feed supplementation with silo, hay, or concentrate, quarantine upon the entry of new animals, presence of diarrhea, and herd size. The variables related to the animal were gender, age group, and breed.

Statistical analysis

The samples of this descriptive cross-sectional study were selected through a simple random sampling method, in which each member of the study population had the same probability of being selected. The apparent prevalence and the real prevalence were determined utilizing the statistical software WinEpi. With the epidemiological database consolidated in Excel, the results were processed by the statistical software EpiInfo®. The determining factors were established by calculating the prevalence ratio, where the dependent variable was the serological results and the independent variables were all the determining factors established in the structured epidemiological applied survey. The association between the variables and the obtained results was examined by Fisher's exact test. Once these factors were established, a final model was built using logistic regression analysis. The size of animals and herds affected by PTB that were exposed to a factor was compared with the same proportion of a population not exposed to that factor to estimate the prevalence ratios. Prevalence ratio was used because this test is recommended for cross-sectional studies, and is a conservative and consistent measure of association with better interpretation. The prevalence ratio was used to evaluate the association between PTB and the determinants, as well as the importance of these associations by the means of Fisher's exact test [54]. Prevalence ratios greater than 1 (95% LCI lower confidence interval < 1) and with $p < 0.05$ were considered risk factors, while prevalence ratios less than 1 (95% UCI upper confidence interval <1) and with $p < 0.05$ were considered protective factors. The dependent variable was the MAP ELISA results, and the independent variables included all the determinants established in the epidemiological survey applied during the sampling, such as age group, breed, gender, and the

management and biosecurity variables of the farm. Once these factors were established, a stratified logistic regression was performed to test for confounding and identify simultaneous interactions between variables significantly associated with MAP [55] (Figure 1).

Ethical considerations

The study was carried out under the regulations of laws 576, 2000, and 84 of 1989 of the Republic of Colombia. Informed consent was obtained from animal owners prior to sample collection.

Authors' Contributions

S.E.C.E conceived and designed the experiment and interpreted the data, D.J.L.B. collaborated with the experiment and critically revised the manuscript. D.M.B.C collaborated with the experiment and analyzed the data. D.J.G.C. critically revised the manuscript. M.O.P.M. supervised the experiment and critically revised the manuscript.

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Competing Interests

The authors declare that there is no conflict of interest.

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Treatment and outcomes of horses with acute synovitis in the racing season: a 167 case series study

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ABSTRACT

Among the causes of lameness in horses, those of arthrogenic origin, especially synovitis, which can progress to advanced joint damages, such as osteoarthritis, are of importance in clinical practice. In this study, we aimed to evaluate the early diagnosis, treatment, and treatment outcomes of acute synovitis, which occur frequently in English and Arabian horses during the racing season. A total of 167 joints of 126 horses (39 English and 87 Arabian Thoroughbred) aged 2-4 years diagnosed with acute synovitis were evaluated using clinical, radiographic, and ultrasonographic examinations. The treatment protocol of horses consisted of cold hydrotherapy and light pressure bandage initially, followed by rest for 10 days and controlled walking only, a single dose of phenylbutazone, and intra-articular corticosteroid and hyaluronic acid injection. Although functional improvement was observed in all the horses following this treatment, subsequent relapses occurred in 22 cases. Consequently, it is important to comply with this treatment program and plan the treatment of acute synovitis in the early period. Controlled gaits, rather than absolute immobility, increase the success of treatment, especially in horses at rest. In addition, it is important to ensure that the relevant joint is not overburdened after the rest period in order to avoid relapses.

Keywords

Joint, Osteoarthritis, Racehorse, Synovitis

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Abbreviations

AAEP: American Association of Equine Practioners
MHz: Megahertz

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Introduction

Similar to many other species, the joints of horses have a structure that absorbs concussion and allows limb movement. The two ends of the bone are enclosed in a fibrous capsule that helps provide stability, and stabilization is achieved by attaching the collateral ligaments to the sides of each of the bones in the joint capsule. The interior of the joint capsule consists of synovial membranes that cover its sides. The synovial membranes produce "synovia" to allow frictionless movement of the joint and are an integral part of the joint. In addition to its lubricating feature, synovial fluid supplies nutrients to the joint and removes waste products from the joint [1, 2, 3]. Moreover, the synovial joints are at great risk because they are the most active joints in a horse's body. They consist of two bone ends covered with the articular cartilage, which is so smooth and flexible, allowing frictionless movement of the joint if properly lubricated [2, 4].

The joints are exposed to stress, pressure, and cyclical trauma daily, especially in racehorses, and the greatest stress is experienced when the horse stops after moving or galloping at high speeds. Wear and tear that can cause joint problems is often caused by such stress and cyclical trauma [2]. Wear and tear usually occurs as a result of the inflammation of the synovial membranes (synovitis), which are very important and contribute to the frictionless movement of the joint [5].

Synovitis is very common in racehorses, and results in serious complications, including osteoarthritis, if not treated effectively [6, 7-9]. Therefore, early diagnosis and treatment of synovitis before the occurrence of any complications that lead to joint damage are important. In most cases, symptomatic treatment is performed with cold hydrotherapy or agents, such as nonsteroidal anti-inflammatory agents. Consequently, the overlooked synovitis progresses further [10, 11].

Therefore, in this study, we aimed to evaluate the early treatment and treatment outcomes of synovitis, which frequently occurs in racehorses. We emphasized the importance of rest and a controlled exercise program together with early treatment for synovitis in horses.

Results

The horses included in the study consisted of only Arabian (n = 87, 69.04%) and English Thoroughbred (n = 39, 30.95%) horses. The included animals were young and aged 2-4 years. English horses were 2-4 years old, while Arabian horses were 3 years old. The gender distribution of the horses is shown in Table 1.

During clinical examination, a healthy posture was observed in some horses when they were inspected in the standing position, whereas in the horses with a high lameness score, the swelling was more pronounced, and the foot touched the ground with the tip of the toe. Similarly, it was observed that these horses did not equally distribute their body weight on their four legs.

Effusion in the affected joints (Figure 1) caused swelling only in certain areas in cases with low effusion scores, whereas in cases with high effusion scores, the joint was entirely swollen and the joint capsule was tense. The effusion scores are presented in Table 2. Mild, moderate, and severe increases in temperature and tenderness were found in the palpation of the joint area. While pain during flexion was less in horses with less inflammatory symptoms and a less degree of effusion, it was quite severe in horses with severe effusion and a high degree of inflammation. The results obtained in the flexion test are presented in Table 2.

All the horses showed a varying severity of lameness and were graded according to the American Association of Equine Practitioners (AAEP) lameness

Figure 1.
A. Swelling appearance due to effusion of the carpal joint. B. Swelling of the tarsal joint due to effusion. C. The gush of synovial fluid during puncture due to loss of viscosity of the synovial fluid. D. Change in aspirated synovial fluid color and loss of viscosity are shown.

score. The data are summarized in Table 2. In the physical examination of the synovial fluid, the color was orange to transparent and had lost its viscosity to a considerable extent (Figure 1). Furthermore, viscosity loss was observed to be much higher, especially in cases with an effusion score of 3-4.

No comparison was made between Arabian and English horses in terms of joint location. It was observed that the bilateral metacarpophalangeal joint was affected in six horses, cross metacarpophalangeal and tarsocrural joints in one horse, distal interphalangeal and metacarpophalangeal joints of the same extremity in one horse, and one tarsocrural joint, one distal interphalangeal joint, and one metacarpophalangeal joint in two horses. It was determined that the most commonly affected joints were the metacarpophalangeal joint of the anterior extremity (n = 86, 51.49%) and the tarsal joint of the posterior extremity (n = 27, 16.16%). Synovitis was most common in the tarsocrural joint in the tarsal joint. Effusion was observed in the tarsometatarsal joint in only one case. Other joints with acute synovitis apart from the abovementioned joints are shown in Table 2.

During the post-treatment follow-up of the horses (Figure 3), the symptoms of local inflammation were observed to disappear within 3-4 days. At the end of the 10-day resting period, all the horses showed a functional improvement in the clinical and lameness examinations, as well as the clinical score (i.e., effusion, lameness, pain score in flexion). However, 21 horses (16.66%) had a relapse of synovitis in the same joint and 29 (23.01%) in different joints. It is important to note that these relapse cases did not comply with the recommended resting schedule and quickly resumed intense exercises after the treatment was completed.

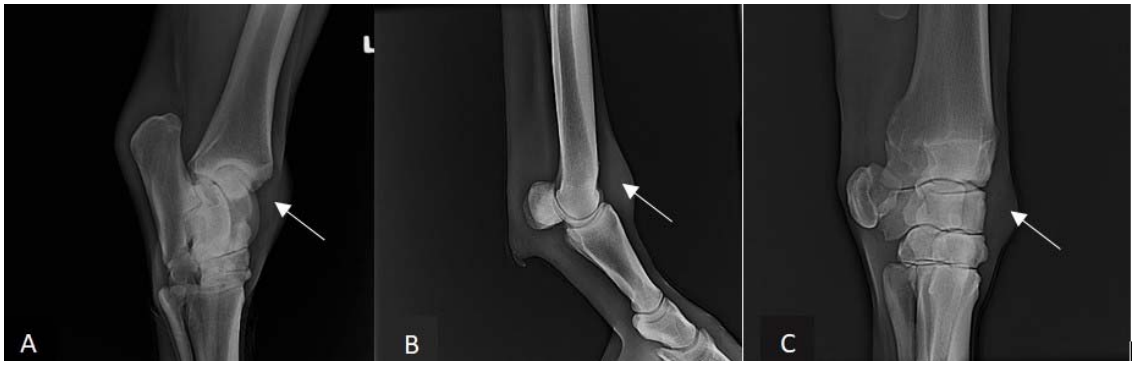

Figure 2.
Radiographs of different cases; A. Synovitis of the tarsocrural joint in a 4-year-old English Thoroughbred. B. Synovitis of the metacarpophalangeal joint in a 4-year-old English Thoroughbred, C. Synovitis of the carpal joint in a 3-year-old Arabian Thoroughbred. White arrow: Tension and swelling in the joint capsule with the developing effusion.

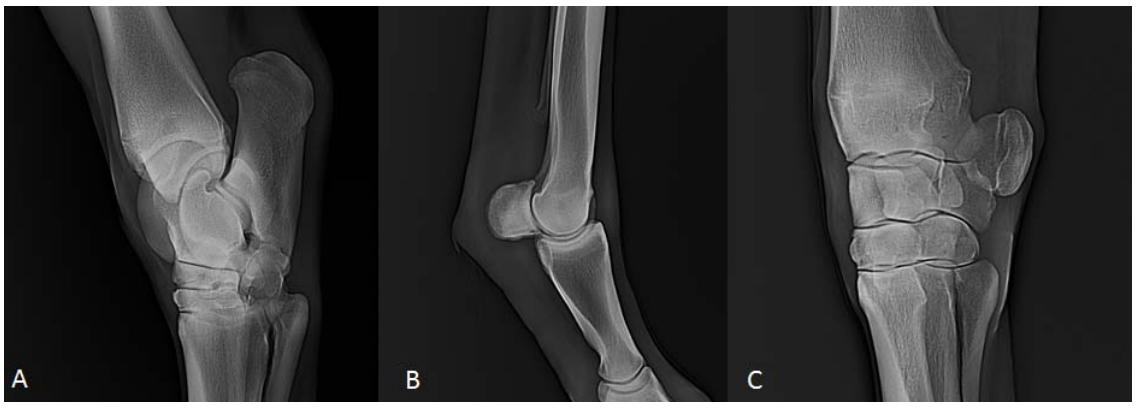

Figure 3.
Post-treatment radiographic images of the cases given in Figure 1.

Table 1.
The affected joints, effusion score, flexion pain score, and lameness score of English and Arabian horses evaluated in the racing season

Horse		The affected joint					Effusion score					Pain score in flexion				Lameness score					
		M1	M2	D	T	C	0	1	2	3	4	0	1	2	3	0	1	2	3	4	5
Arabian horses	Y: 87 (50 F, 37 M)	53	15	5	18	16		3	56	43	5		7	34	66		4	25	30	43	5
English horses	Y: 39 (19 F, 20 M)	33	6	5	9	7		4	27	28	1		4	24	32		2	14	11	11	1
Total	126 (69 F, 57 M)	86	21	10	27	23		7	83	71	6		11	58	98		6	39	41	54	6

Y: Young (0-4 years), F: Female, M: Male.
The affected joint: M1: Metacarpophalangeal joint, M2: Metatarsophalangeal joint, D: Distal interphalangeal joint, T: Tarsal joint, C: Carpal joint
Effusion score: 0: No effusion, 1: Mild, 2: Moderate, 3: Severe effusion, 4: Severe swelling of the joint region
Pain score in flexion: 0: No pain, 1: Mild pain, 2: Moderate pain, 3: Severe pain
Lameness score: 0: Lameness not perceptible under any circumstances. 1: Lameness is difficult to observe and is not consistently apparent, regardless of circumstances (e.g. under saddle, circling, inclines, hard surface, etc.). 2: Lameness is difficult to observe at a walk or when trotting in a straight line but consistently apparent under certain circumstances (e.g. weight-carrying, circling, inclines, hard surface, etc.). 3: Lameness is consistently observable at a trot under all circumstances. 4: Lameness is obvious at a walk. 5: Lameness produces minimal weight bearing in motion and/or at rest or a complete inability to move.

Discussion

Joint pain is one of the causes of lameness in performance horses [13]. Traumatic arthritis, which is one of the common joint diseases in horses, includes a combination of various pathological and clinical symptoms. Acute arthritis that develops after experiencing a single trauma or repeated traumas entails 1) synovitis (inflammation of the synovial membrane), 2) capsulitis (inflammation of the joint capsule), 3) sprains (injury of certain ligaments), 4) intra-articular fractures, and 5) meniscal tears (femorotibial joints). The presence of one of these conditions alone can also lead to osteoarthritis progression [14, 15]. Therefore, early diagnosis and effective treatment of acute synovitis are very important for preventing permanent damage to the joint [15, 16]. The present study aimed to emphasize the importance of rest and controlled exercise together with the early treatment of common synovitis in racehorses.

Two basic pathobiological processes should be considered in the case of a traumatically injured joint. The first of these is the inflammation of the synovial membrane and joint capsule (synovitis and capsulitis) and the other is biochemical damage to the articular cartilage and bones [4, 14]. In the case of acute synovitis and capsulitis, some enzymes and cytokines are released (e.g., matrix metalloproteinases, aggrecanases, prostaglandins, free radicals, tumor necrosis factor-alpha, and interleukin-1), which lead to synovial membrane inflammation, important clinical symptoms, and synovial structure disruption, which in turn result in effusion [3, 6, 8, 14, 17, 18]. Minimizing joint damage and stopping the progression of acute inflammations of the synovial membrane and joint capsule are important [13, 15]. Otherwise, serious complications, including articular cartilage lesions and osteoarthritis may occur [8, 13, 18]. The horse owners or caregivers were informed about the importance of early treatment, adequate rest, and a gradual exercise program in all horses included in this study. However, recurrence (n = 21, 16.16%) was observed in horses that did not comply with adequate rest and a gradual exercise program. Recurrence and such disruptions in the treatment process will predispose to osteoarthritis by creating disorders in the anatomical structures of the joint in the following periods.

The clinical approach towards treating a horse with lameness or joint swelling is important and requires a good clinical examination to determine whether or not the underlying problem is primarily in the soft tissues. In cases where synovitis is suspected, radiographs are often used to evaluate the joint for determining the presence of osteochondral damage. In addition, further radiographs should be performed to identify changes suggestive of further structural damage in chronic cases that do not respond to treatment. Moreover, synovial fluid analysis (at least physical examination) is always useful [14, 16, 19]. Colour and viscosity assessments can be performed in the synovial fluids aspirated under aseptic conditions. Synovial fluid analysis should be completed to rule out infective arthritis, especially in severe lameness associated with synovial effusion [3, 16]. In this study, after a detailed medical history was recorded for each horse presenting with the

complaint of lameness and swelling in the joint area, the location of the lesion was determined according to the findings during inspection and palpation. Furthermore, radiographs were obtained and evaluated for the presence of other possible damages. In addition, an ultrasonographic examination was performed to determine the surrounding soft tissue damage. Damage to the bone tissue or other joint tissues, such as the surrounding capsule and ligament, was evaluated. Radiographic and ultrasonographic examinations were found to be very useful in distinguishing cases with only acute synovitis from other cases. The physical examination of the synovial fluid revealed that all the horses had varying degrees of viscosity loss. Synovial viscosity loss was more severe in horses with severe effusion, and pain during flexion and lameness was more severe in these horses. This has been attributed to pain and effusion due to inflammation. Therefore, treatment aims to reduce effusion and relieve pain along with management of inflammation. In the treatment method described in this study, cold hydrotherapy application and aspiration of the excess fluid in the joint were performed, followed by intra-articular corticosteroid administration. Next, the joint was protected with an animalintex bandage. When the signs of inflammation subsided, hyaluronic acid was injected into the joint. This treatment protocol was complementary and highly effective in cases of acute synovitis. However, the occurrence of relapses was found to be significant in horses that did not take adequate rest after the treatment or started high-intensity training within a short time post-treatment. The data obtained from this study showed that initiating a subsequent training process after the treatment protocol would be beneficial when sufficient time is passed.

In the treatment of synovitis in horses, nonsteroidal anti-inflammatory agents, cold hydrotherapy, and hyaluronic acid are applied [10, 11, 15]. Grauw et al. [7] emphasized that phenylbutazone as a nonsteroidal anti-inflammatory agent is clinically effective in cases with acute synovitis and does not limit cartilage catabolism, but can temporarily reduce collagen anabolism. McIlwraith et al. [20] reported at the 17th Kentucky Equine Research Nutrition Conference that cold hydrotherapy delays inflammatory processes, such as exudation and diapedesis in acute joint injuries and is effective in reducing edema. In this study, a single dose of slow intravenous phenylbutazone injection was administered together with cold hydrotherapy and intra-articular corticosteroid injection, and swelling and inflammation in the joint area decreased in all cases.

In many investigations, intra-articular corticosteroids have been recommended in the treatment of acute synovitis [5, 15, 18, 21], namely betamethasone, triamcinolone acetonide, and methylprednisolone acetate. It has been reported that triamcinolone acetonide increases the concentration of hyaluronic acid and glycosaminoglycan in the synovial fluid and reduces total protein and inflammatory cell infiltration. Methylprednisolone is a long-acting compound compared to betamethasone and triamcinolone acetonide. However, it has been indicated to cause chondrocyte necrosis and proteoglycan loss [21]. As a result, triamcinolone acetate was preferred as the intra-articular corticosteroid in this study. McIlwraith et al. [20] emphasized that there was a significant improvement in the degree of lameness, morphological parameters of synovial fluid, synovial membrane, and articular cartilage in horses treated with triamcinolone acetate. Triamcinolone acetonide was administered to all horses included in this study and all recovered functionally.

Extremity joints of racehorses are subject to stress and cyclic trauma due to racing and intense exercise. The wear and tear that can damage the joints is often caused by such stress and cyclic trauma [2]. Wear and tear on the joint surface is usually caused by the inflammation of the synovial membranes (synovitis) [5]. Hyaluronan is a glycosaminoglycan that plays an important role in the formation of proteoglycan aggregates in articular cartilage. It is also a component of synovial fluid and indirectly contributes to the viscosity of the synovial fluid. In addition, intra-articular hyaluronan has a pain-relieving effect [12]. Niemela et al. [12] reported that a single dose of intra-articular hyaluronic acid injection significantly reduced pain scores in joint effusion and flexion and that it could be used to diminish lameness in synovitis and mild osteoarthritis. In our study, intra-articular hyaluronic acid was injected into all horses diagnosed with synovitis, and functional improvement was achieved in all of them. It was thought that the later recurrence was due to a rapid transition to rest and an intense exercise program.

Taking rest has a clear benefit in cases of acute synovitis and capsulitis. It may not be possible to provide rest to horses engaged in racing or other athletic activities and allow for a full recovery. Bandage support can also help an acutely damaged joint heal. In such cases, it has been shown that a light pressure bandage stimulates the mechanoreceptors, which may reduce the sense of pain [15]. Immobilization is important in traumas, but it is not that important if the problem is limited to synovitis/capsulitis. This is because long-term immobilization can cause muscle atrophy, adhesion formation, and articular cartilage atrophy in the joint. Passive flexion of the limbs and joints can help maintain mobility, and controlled gait exercises are recommended in most cases. In

addition, intense exercises, such as training, should be discontinued and only controlled gait exercises should be continued instead. This would maintain the movement of the joint capsule and prevent atrophic changes in the articular cartilage [5, 15]. In all horses treated in this study, a light pressure bandage was applied to the joint on the first day. After 3 days of stable rest, 7 days of controlled walks and 10 days of rest were recommended. It is an important finding that relapses occur in horses that do not fully comply with this resting schedule.

Acute synovitis often occurs in racehorses or athletic horses and progresses into articular cartilage degeneration or osteoarthritis over time due to the recurrences of trauma or stress factors [10]. Osteoarthritis mostly occurs in the middle and old ages [3]. The horses included in the current study were young horses aged 2-4 years. These horses in question will be predisposed to osteoarthritis in their later years if they are not treated appropriately and if they do not comply with adequate rest and controlled exercise programs.

In young horses, synovitis is most common in the metacarpophalangeal joint of the anterior limbs [6, 12, 14], followed by the carpal joints [6, 14]. The tarsal joint is a common etiology of posterior limb lameness in horses. The proximal intertarsal joint is associated with the tibiotarsal joint; therefore, lesions of the proximal intertarsal joint can often be observed in the tarsal joint, which is a highly mobile weight-bearing joint [22]. In this study, considering the location of the affected joints, it is noteworthy that cases of synovitis were more common in the anterior extremities, especially in the metacarpophalangeal joint (51.49%) (Table 2). This may be related to the fact that the body weight on the anterior extremity is higher and the resulting stress increases. Synovitis cases were more common especially in the tarsocrural joint (16.16%) among the joints of the posterior extremity, which can be attributed to the high mobility of this joint and the great stress experienced by that joint during movement.

In conclusion, it is important to follow the treatment schedule and plan an early treatment of acute synovitis, which often occurs in racehorses. Controlled gaits, instead of absolute immobility, increase the success of treatment in resting horses, and in order to avoid relapses, the relevant joint should not be overburdened as soon as the rest period is over. Furthermore, acute swelling in the joint area should not be considered acute synovitis, and radiographic and ultrasonographic examinations should be conducted for differential diagnosis.

Table 2.
Scorings used during clinical examination

Effusion score		Pain score in flexion		AAEP* lameness scale	
0	No effusion	0	No pain	0	Lameness not perceptible under any circumstances
1	Mild effusion	1	Mild pain, i.e. the horse shows some reaction, such as moving the limb	1	Lameness is difficult to observe and is not consistently apparent, regardless of circumstances (e.g. under saddle, circling, inclines, hard surface, etc.)
2	Moderate effusion	2	Moderate pain, i.e. the horse retracts the limb repeatedly during the 1 min flexion period	2	Lameness is difficult to observe at a walk or when trotting in a straight line but consistently apparent under certain circumstances (e.g. weight-carrying, circling, inclines, hard surface, etc.)
3	Severe effusion	3	Severe pain, i.e. the flexion test cannot be properly performed	3	Lameness is consistently observable at a trot under all circumstances
4	Severe swelling of the joint region			4	Lameness is obvious at a walk
				5	Lameness produces minimal weight bearing in motion and/or at rest or a complete inability to move

*AAEP: American Association of Equine Practitioners

Materials & Methods

The study material consisted of 167 joints of 126 horses belonging to different races and genders with an age range of 2-4 years. They were brought to the Turkey Jockey Club Elazıg Horse Hospital in the 2018 racing season (April-October). Permission for the study was obtained from Dicle University Animal Experiments Ethics Committee with the document numbered E-35582840-604.01.01-133490.

An orthopedic examination was performed after a thorough medical history was recorded for each horse that presented with complaints of swelling in the joint area (joints are given in Table 2) and lameness. Temperature, crepitation, and tension in the joint capsule were evaluated by palpating the swollen joint. In addition, horses were observed during normal gait and trot movements. Radiographic and ultrasonographic examinations (10-18 MHz probe) were performed in various aspects (dorsopalmar, dorsoplantar, lateromedial, mediolateral, mediolateral in flexion position, dorsolateral-palmaromedial oblique, dorsomedial-palmarolateral oblique, and skyline views) to ensure that there was no damage to the bone and soft tissues in the joint area. Horses showing lesions (desmitis, tendon rupture, degenerative joint damage, fracture, dislocation, and osteochondritis dissecans) in the radiographic (Figure 2-3) and ultrasonographic examinations were not included in the study. In addition, the synovial fluid (Figure 1) was physiologically examined by puncturing the swollen joint. The leukocyte count in the synovial fluid was measured using a blood count device. Cases with particles or increased leukocyte count in the synovial fluid were excluded from the study. Moreover, effusion scoring, flexion pain scoring, and American Association of Equine Practitioners (AAEP) lameness scoring [12] were performed for all horses included in the study (Table 2). Relapses in the same joint were not evaluated within the scope of these scorings.

After performing all the examinations, cold hydrotherapy and a light pressure bandage were applied initially, and a 10-day resting period was advised with daily controlled gaits during this resting period in the horses diagnosed with synovitis. Each horse received phenylbutazone (Equi-Butazon®, Provet, Turkey) as a slow intravenous infusion single dose of 4 mg/kg and was bandaged with a poultice pad (Animalintex® poultice dressing, Robinson Healthcare, England) for 2 days. In addition, intra-articular triamcinolone acetonide (Sinakort A®, 40 mg, İbrahim Etem, Turkey) was administered to each relevant joint. The patients were monitored daily and intra-articular hyaluronic acid (Regenflex®, 2 ml, Intrafarma, Italy) was injected when the severity of inflammation (temperature and pain) in the joint area declined. The horses were monitored through routine follow-ups until functional recovery was achieved.

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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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The effect of Artemisinin on the Pentylentetrazole-induced seizures during the estrous cycle and GABA interaction in mice

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ABSTRACT

Catamenial epilepsy may involve 10 to 70% of women with epilepsy in which, seizures are exacerbated by the menstrual cycle. Artemisinin is a herbal compound with widespread modern and traditional medical indications. Because of GABAergic interaction, this study was designed to study the antiepileptic effects of Artemisinin during the estrus cycle. A total of 360 adult female mice were placed in 10 groups: control, solvent (ethanol 10ml/kg), Artemisinin (75&150 mg/kg), Bicuculline (2mg/kg), Bicuculline (2mg/kg) + Atremisinin (75&150 mg/kg), Saclofen (2mg/kg), Saclofen (2mg/kg) + Atremisinin (75&150 mg/kg), each with four subgroups (proestrus, estrus, metestrus and diestrus) (n=9). One week after acclimatization, estrous synchronization and phase determination was achieved. Acute epilepsy was induced by intraperitoneal (i.p) injection of 80 mg/kg of Pentylentetrazole (PTZ), 30 minutes after i.p injection of Artemisinin and ethanol. Initiation time of myoclonic seizures (ITMS), initiation time of tonic-clonic seizures (ITTS), seizure duration (SD), and mortality rate (MR) were recorded for 30 minutes. Data were displayed as mean ± SD and evaluated using one-way ANOVA followed by Tukey–Kramer multiple comparison post hoc tests ($p < 0.05$). Artemisinin significantly decreased epilepsy incidence, duration, and mortality rate, in parallel to increasing ITMS and ITTS in a dose-dependent manner which were more prominent during the luteal phase. Co-administration of Biccucluline significantly inhibited antiepileptic effects of Artemisinin, while Saclofen did not have such an inhibitory interaction. It seems that increased neurosteroid metabolites and GABAA receptors, neural hyperpolarization following GABA interaction, and anti-inflammatory and anti-oxidative properties which decrease neuroinflammation and neural excitability can participate in the antiepileptic effects of Artemisinin.

Keywords

Artemisinin, catamenial epilepsy, estrous cycle, GABA

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Abbreviations

AEDs: Antiepileptic drugs
AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid AP: Allopregnanolone
ART: Artemisinin

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Introduction

Epilepsy is one of the most common and classical CNS diseases, defined as unpredictable repetitive seizures, which may involve 0.5-1% of the people in industrial countries. Epilepsy affects life quality and social relations of the patients. In 10 to 70% of women with epilepsy, particularly those with focal or generalized types, a cyclic model of seizure exacerbation aligns with the menstrual cycle, especially around the perimenstrual or the periovulatory period has been seen. Thus, based on the Greek word katomenios or monthly, it has been called catamenial epilepsy. Sex steroids and their metabolites play a critical role in seizure initiation and propagation via contributing to the development and organization of the CNS and neural excitability since progesterone exerts anticonvulsant while estrogens exert proconvulsant characteristics [1,2,3,4,5]. Despite numerous antiepileptic drugs (AEDs) which can control epilepsy in about 60% of patients, catamenial epilepsy remains a drug-resistant form [6]. Because of the side effects of AEDs such as hepatotoxicity, an increased desire for natural remedies and safer treatments such as herbal ones are unavoidable. Artemisinin is an endoperoxide sesquiterpene lactone isolated from Artemisia annua that has wide-ranging folkloric and contemporary remedial indications e.g. anti-malarial, anti-fungal, anti-bacterial, anti-leishmania, anti-coccidian, anti-diabetic, anti-spasmodic, anti-oxidative, anti-inflammatory, analgesic, and wound healing [7]. Artemisia species contain considerable sources of flavonoids, which can act as positive allosteric modulators of the GABAA receptor, so display anxiolytic, sedative, and antinociceptive actions [7]. Artemisinin interacts with the Gephyrin part of the GABAA receptor complex, activates it, increases GABA signaling, and inhibits nociceptive signaling in the dorsal root ganglia leading to analgesia [8,9]. Artesunate can protect against maximal electroshock and PTZ-induced seizures in mice [10]. Basal levels of progesterone in the brain can alter GABA receptor density throughout the estrous cycle [11]. Based on these findings and fluctuating levels of steroid hormones and their metabolites along the estrous cycle, this study was planned to investigate the effect of Artemisinin on the PTZ-induced seizures during the estrous cycle and the possible role of the GABAergic system.

Abbreviations-Cont'd

BIC: Bicucculine
GABA: Gama aminobutyric acid
ITMS: Initiation time of myoclonic seizures
i.p.: intraperitoneal
ITTS: Initiation time of tonic-clonic seizure
MR: mortality rate
NMDA: N-Methyl-D-aspartate
PTZ: Pentylentetrazole
SAC: Saclofen

Results

Seizure duration

Artemisinin significantly decreased seizure duration through the estrous cycle, dose-dependently ($p < 0.05$). There was no significant difference between Saclofen, Bicucciline, Bisucculine plus Artemisinin and control ($p > 0.05$). While the difference between the Artemisinin and Artemisinin-Saclofen combination was not significant ($p > 0.05$), Saclofen + Aartemisinin significantly lowered seizure duration than that in the control group ($p <0.05$). In all treatment groups, seizure duration was significantly lower in the luteal phase than in the follicular phase ($p < 0.05$) (Table 1).

Mortality rate

Artemisinin administration, significantly and dose-dependently decreased the mortality rate in comparison to that in the control group, all along the estrous cycle ($p < 0.05$). There was no significant difference between groups that received Saclofen, Bicucciline, Bisucculine plus Artemisinin, and the control group ($p > 0.05$), but Salcofen + Artemisinin co-administration significantly decreased the mortality rate in comparison to the control ($p < 0.05$). In all groups, the mortality rate was significantly lower during the luteal phase than in the follicular phase ($p < 0.05$) (Table 2).

ITMS

Artemisinin significantly and dose-dependently increased ITMS, ($p < 0.05$), which in all groups, it was significantly higher during the luteal phase than in the follicular phase ($p < 0.05$). There was no significant difference between groups that received Saclofen, Bicucciline, Bisucculine plus Artemisinin, and the control group ($p > 0.05$), but Salcofen + Artemisinin co-administration significantly decreased ITMS in comparison to the control group ($p > 0.05$) during the entire estrous cycle (Figure 1).

ITTS

ITTS was significantly increased by Artemisinin in a dose-dependent way. In all groups, it was significantly higher during the luteal phase than the follicular phase ($p < 0.05$). In all phases of the estrous cycle, there was no significant difference between groups that received Saclofen, Bicucciline, Bisucculine plus Artemisinin, and the control group ($p > 0.05$), but Sal-

cofen + Artemisinin co-administration significantly decreased ITTS in comparison to the control group (Figure 2).

Discussion

To the authors' knowledge, this is the first study about the effect of Artemisinin on PTZ-induced seizures during the estrous cycle and GABAergic interaction. Decreasing seizure incidence, duration, and mortality rate in addition to increasing ITTS and ITMS indicated antiepileptic properties of Artemisinin, that through the luteal phase, it was markedly more than the follicular phase. These antiepileptic properties can be mediated by the GABAA recep-

Table 1.
Effects of Bicuculline (BIC; 2mg/kg), Saclofen (2 mg/kg), Artemisinin (ART; 75 and 150 mg/kg) and combination of BIC (2 mg/kg) and Saclofen (2 mg/kg) with ART (75 and 150 mg/kg) on seizure duration (SD) (sec) during various phases of the estrous cycle.

Group/Estrous Cycle	Proestrous	Estrous	Metestrous	Diestrous
Control	771 ± 41^{a}	752 ± 53^{a}	507 ± 41^{a*}	513 ± 39^{a*}
Vehicle	753 ± 37^{a}	731 ± 34^{a}	531 ± 41^{a*}	524 ± 36^{a*}
BIC (2mg/kg)	801 ± 72^{a}	793 ± 64^{a}	578 ± 38^{a*}	584 ± 53^{a*}
Saclofen (2 mg/kg)	741 ± 63^{a}	712 ± 65^{a}	491 ± 36^{a*}	497 ± 31^{a*}
ART (75 mg/kg)	570 ± 43b	527 ± 39b	401 ± 37^{b*}	366 ± 35^{b*}
ART (150 mg/kg)	374 ± 33c	392 ± 46c	237± 25c *	218 ± 25^{c*}
BIC (2 mg/kg) + ART (75 mg/kg)	731 ± 34^{a}	722 ± 64^{a}	536 ± 34^{a*}	505 ± 35^{a*}
BIC (2 mg/kg) + ART (150 mg/kg)	701 ± 58^{a}	729 ± 71^{a}	536 ± 56^{a*}	572 ± 51^{a*}
Saclofen (2 mg/kg)+ ART (75 mg/kg)	552 ± 34^{b}	518 ± 28^{b}	418 ± 31^{b*}	356 ± 27^{b*}
Saclofen (2 mg/kg)+ ART (150 mg/kg)	361 ± 47^{c}	406 ± 54^{c}	208 ± 45^{c*}	215 ± 49^{c*}

Different letters (a, b, or c) in each column indicate a significant difference ($p < 0.05$) between various treatments in each phase of the estrous cycle. Asterisk (*) indicates a significant difference for each phase of the estrous cycle Vs the control group ($p < 0.05$). Data are presented as mean ± SEM.

Table 2.
Effect of Bicuculline (BIC; 2 mg/kg), Saclofen (2 mg/kg), Artemisinin (ART; 75 and 150 mg/kg) and combination of BIC (2 mg/kg) and Saclofen (2 mg/kg) with ART (75 and 150 mg/kg) on the mortality rate (MR) of seizures (%) during various phases of the estrous cycle.

Group Estrous Cycle	Proestrous	Estrous	Metestrous	Diestrous
Control	33.3^{a}	33.3^{a}	16.6^{*a}	16.6^{*a}
Vehicle	33.3^{a}	33.3^{a}	16.6^{*a}	16.6^{*a}
BIC (2 mg/kg)	33.3^{a}	33.3^{a}	16.6^{*a}	16.6^{*a}
Saclofen (2 mg/kg)	33.3^{a}	33.3^{a}	16.6^{*a}	16.6^{*a}
ART (75 mg/kg)	16.6^{b}	33.3^{a}	16.6^{*a}	16.6^{*a}
ART (150 mg/kg)	0^{c}	0^{b}	0^{b}	0^{b}
BIC (2 mg/kg) + ART (75 mg/kg)	33.3^{a}	33.3a	16.6^{*a}	16.6^{*a}
BIC (2 mg/kg) + ART (150 mg/kg)	33.3^{a}	33.3a	16.6^{*a}	16.6^{*a}
Saclofen (2 mg/kg) + ART (75 mg/kg)	16.6^{b}	33.3^{a}	16.6^{*a}	16.6^{*a}
Saclofen (2 mg/kg) + ART (150 mg/kg)	0^{c}	0^{b}	0^{b}	0^{b}

Different letters (a, b, or c) in each column indicate significant differences at the $p < 0.05$ level between various treatments in each phase of the estrous cycle. Asterisk (*) indicates a significant difference for each phase of the estrous cycle Vs the control group ($p < 0.05$).
Data are presented as mean ± SEM.

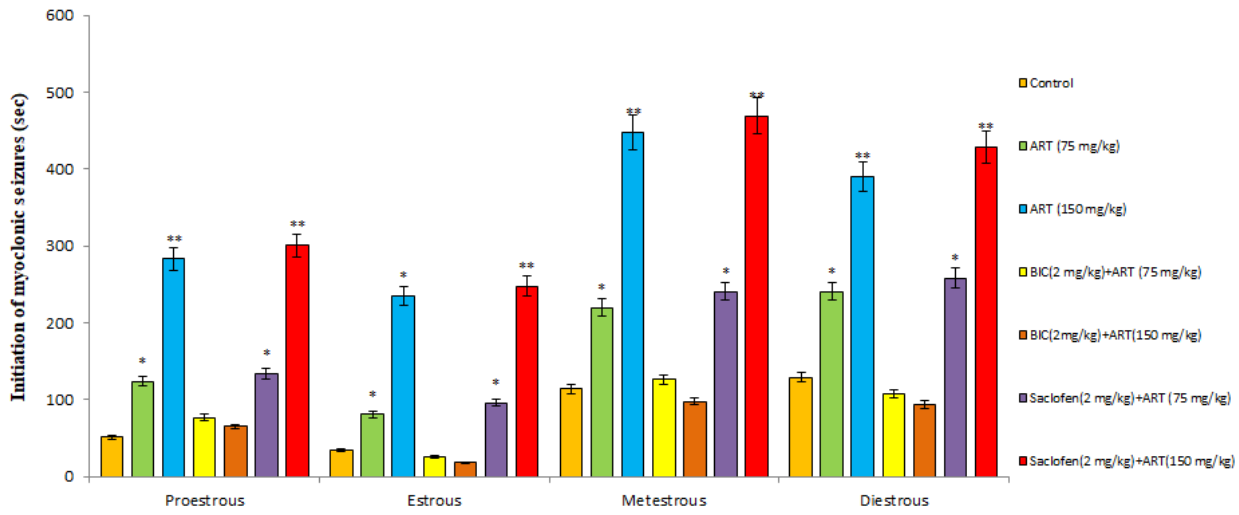

Figure 1.
Effects of Artemisinin (ART; 75&150 mg/kg), Bicuculline (BIC; 2mg/kg), Saclofen (SAC, 2 mg/kg) and combination of BIC (2 mg/kg), SAC (2 mg/kg), and (ART; 75 & 150 mg/kg) on the initiation time of myoclonic seizures (ITMS) (sec) during the estrous cycle. *Asterisks indicate a significant difference in each phase of the estrous cycle compared with that in the control group ($p < 0.05$). Data are presented as mean ± SEM.

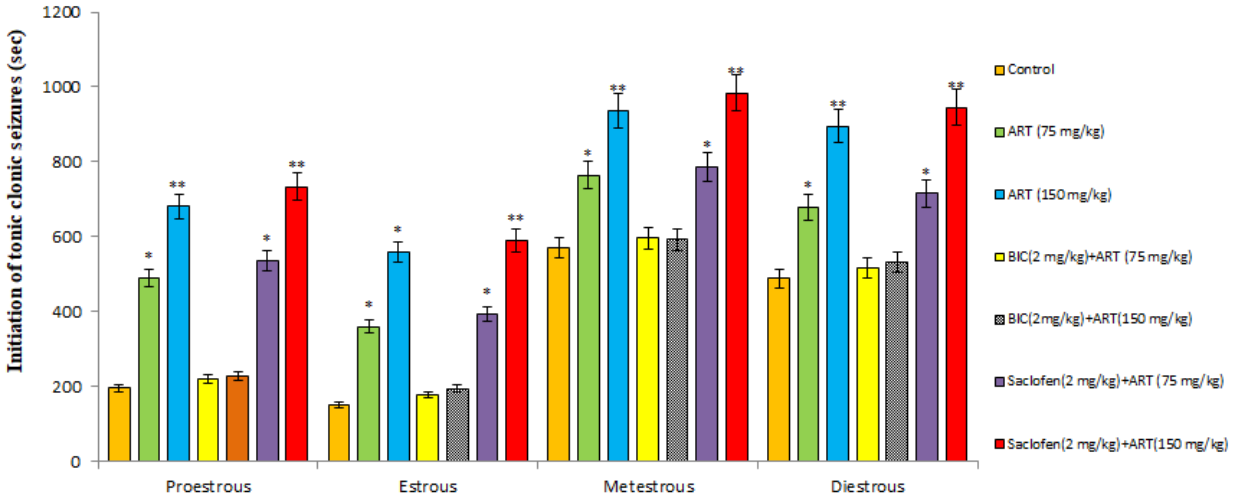

Figure 2.
Effects of Artemisinin (ART; 75&150 mg/kg), Bicuculline (BIC; 2mg/kg), Saclofen (2; mg/kg) and combination of BIC (2 mg/kg), SAC (2 mg/kg), and ART (75 & 150 mg/kg) on the initiation time of tonic-clonic seizures (ITTS) (sec) during the estrous cycle. *Asterisks indicate a significant difference in each phase of the estrous cycle compared with that in the control group ($p < 0.05$). Data are presented as mean ± SEM.

tors since GABAA receptors blocked by Bicuculline inhibited antiepileptic effects of Artemisinin while GABAB receptors blocked by Salcofen did not interfere with these effects.

Periodical variation in the circulating level of estrogens and progesterone through the estrous cycle may engage in the pathophysiology of catamenial epilepsy. Sex steroids and their metabolites govern the synthesis, discharge, and transport of different neurotransmitters and exert significant function in the pathophysiology of seizures. Estrogens are proconvulsant by repressing GABA synthesis and amplitude of GABA-mediated inhibitory postsynaptic current while augmenting the dendritic spines and NMDA synapses on the hippocampus CA1 pyramidal neurons, increasing glutamatergic transmission which enhances neural excitability [1,2,5]. The direct interrelationship between estrogen level and estrogen/

progesterone proportion with seizure vulnerability indicates maximum vulnerability and exacerbation during the premenstrual and periovulatory periods. Perovulatory aggravation may be owing to an estrogen upsurge in the mid cycle, nearly, uncontested by the progesterone before the luteal phase, while, during the premenstrual phase, a lower level of progesterone than estrogen can assist periovulatory seizure exacerbation [1,2,4,5]. Meanwhile, there is a negative correlation between progesterone level and catamenial epilepsy, so that, rapid fall in the progesterone level just before, during menstruation, and then after, is correlated with an increase in catamenial seizures. Progesterone potentiates the inhibitory action of adenosine, decreases neural firing, epileptiform discharges, estrogen receptors, and counteracts estrogen proconvulsant effects via metabolization to the neurosteroid metabolite and enhancement of GABA-mediated inhibition [5,6,12,13]. Neurosteroids have been discovered primarily in some brain divisions such as the hippocampus and neocortex, adjusting various neurotransmitter signaling systems, including GABAA receptors, membrane progesterone receptors, NMDA receptors, and L or T type calcium channels, and protect against different models of seizure. Neurosteroids with a 3α-hydroxy, 5α-reduced structure such as Allopregnanolone (AP) are positive allosteric modulators of GABAA receptors that increase cell surface expression of GABAA receptors, meanwhile, they are negative allosteric modulators of NMDA and AMPA excitatory receptors [4,12,13,14,15,16,17]. Additionally, at higher concentrations, they can activate GABAA receptors directly, and increase the frequency and opening time of chloride channels, therefore, rapidly diminishing neural excitability [4]. GABAA receptors are the most important and abundant inhibitory receptors in the CNS, which induce neural hyperpolarization and dampen neural excitability [7]. Suppressing the neuroprotective effects of AP by GABAA antagonists proves the GABAergic mechanism of action of the neurosteroids [16]. In the female rats, AP shows higher antiepileptic properties during diestrous than estrous, probably due to the progesterone-induced enhancement of GABAA receptor and neurosteroid metabolite production [1,2]. Artemisinin inhibits morphological changes of hippocampus neurons, induces glutamic acid decarboxylase enzyme, and increases GABA level, which protects against sodium penicillin-induced seizures [15]. It interacts with the Gephyrin segment, triggers GABAA receptors, and enhances GABA singling which can be blocked with GABAA antagonists. Gephyrin is a part of the GABAA receptor that exerts an essential role in the distribution of GABAA and glycine receptors to the cell membrane, therefore playing a cardinal function in their inhibitory action [8,9]. Artemisinin interaction with GABAA receptors results in CNS depression and analgesia [7]. Artesunate restricts the seizure spread in the maximal electroshock (MES)-induced seizure by inhibiting Na+ channels and/or glutamatergic excitation via NMDA receptors, so may be helpful in the management of generalized tonic-clonic and partial seizures [10]. Progesterone regulates and increases GABA receptor density in the cerebral cortex, hippocampus, and hypothalamus during the luteal phase, where they act presynaptically to increase GABAergic while modulating glutamatergic release, also inducing G protein-mediated late inhibitory postsynaptic potential [11,18,19]. Bicuculline is a competitive antagonist of the ionotropic GABAA receptors in the hippocampal or cortical neurons, and blocks inhibitory action on the target neurons leading to epilepsy, while Saclofen is a competitive antagonist for the metabotropic GABAB receptors [20]. In this study, Bicuculline inhibited the antiepileptic actions of Artemisinin on the SD, MR, ITTS, and ITMS, while administration of Saclofen did not show such an inhibitory effect, so, it can be concluded that the anti-epileptic effect of Artemisinin is mediated by the GABAA receptors.

There is a direct relation between ovarian progesterone and neurosteroid level so that, due to common metabolic pathways, the enzyme-inducing property of Artemisinin can increase AP concentration and therefore enhances the anticonvulsant effect during the luteal phase. It is in line with a previous report about higher antiepileptic effects of enzyme-inducing AEDs such as carbamazepine, during the luteal phase of the estrus cycle [21].

Artemisinin showed a significant antiepileptic effect against PTZ-induced seizures along the estrous cycle, especially during the luteal phase. It seems that induction of progestin metabolism, increasing neurosteroids level, interaction with GABAA receptors, and neural hyperpolarization in addition to anti-inflammatory and anti-oxidative features decrease neuroinflammation and neural excitability that can lead to the antiepileptic effects of Artemisinin.

Materials & Methods

Animals and experimental design

360 adult female albino N-MRI mice weighing 25-30 grams were received from the University animal house and kept under established conditions, according to the European community protocols for the protection of animals used for scientific purposes with freely available fresh, clean water, and chow pellets. Experiments were carried out following the guidelines for the care and use of laboratory animals to investigate experimental pain in animals [22], approved by The University Research Ethics Committee (97GRN1M1904). After one week of familiarization, animals were split coincidentally into 10 groups

(n = 9) and 4 trials. At first, by examination of the vaginal smears, sexual puberty was determined. The estrous cycle phase was decided based on the chief cell of the vaginal smears, and mice with two regular estrous cycles were chosen. Then, estrous synchronization was set [3,21]. Experiment 1 was achieved to study the effect of Artemisinin on the PTZ-induced seizures during the proestrus phase. The seizure was induced by i.p. injection of 80 mg/kg of PTZ (Sigma-Aldrich, USA) 30 minutes after i.p. administration of 75 and 150 mg/kg Artemisinin (Alexis Biochemicals, USA) and 10ml/kg of ethanol as solvent (Merck, Germany) (Table 3). Then, animals were observed for 30 minutes and antiepileptic parameters including ITTS, ITMS, MR, and SD were charted. Other experiments were done similarly to evaluate the effects of Bicuculline, GABAA antagonist (BIC; 2mg/kg, Sigma-Aldrich, USA), Saclofen, GABAB antagonist (SAC, 2 mg/kg, Sigma-Aldrich, USA), and a combination of BIC (2 mg/kg) and SAC (2 mg/kg) with ART (75 & 150 mg/kg) during estrous, metestrous, and diestrous phases. For prevention of the likely effect of circadian rhythm on seizure susceptibility, all experiments were fulfilled between 9 am to 3 pm [3,21]. Finally, the animals were euthanized by i.p administration of a high dose of Sodium thiopental (Novartis, Switzerland).

Statistical analysis

Data are summarized as mean ± SEM and analyzed by one-way analysis of variances (ANOVA) followed by Tukey–Kramer multiple comparison post hoc tests ($p < 0.05$).

Table 3.
Treatment procedure in Experiment 1.

Group	1st injection	2nd injection*
Control	*Normal saline*	PTZ (80 mg/kg)
ART (75 mg/kg)	ART (75 mg/kg)	PTZ (80 mg/kg)
ART (150 mg/kg)	*ART (150 mg/kg)*	PTZ (80 mg/kg)

*30 min after the first injection; Pentylenetetrazol (PTZ); Artemisinin(ART)

Authors' Contributions

J.K.: Study design, final manuscript preparation; M.Z.: Statistical analysis; S.H.D.: primary manuscript preparation; M.B.: animal study and data collection.

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Competing Interests

The authors declare that they have no conflict of interest.

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مروری بر گونه های بابزیا و کنه های ناقل در حیوانات ایران

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چکیده

بابزیوز یکی از بیماری های مهم منتقله از کنه ها در کشور های گرمسیری و نیمه گرمسیری بوده که طیف وسیعی از حیوانات اهلی، وحشی و گاهی انسان را تحت تاثیر قرار می دهد. تاکنون بیشتر از صد گونه بابزیا درحیوانات شناسایی شده است. ایران یکی از بزرگترین کشور های خاور میانه، با داشتن دو سلسله جبال زاگرس و البرز آب و هوای متنوعی با فون گیاهی و جانوری شده که زمینه مساعدی برای تکثیر کنه های ناقل تک یاخته های خونی مثل بابزیا و تیلریا فراهم نموده است. در دهه گذشته؛ مطالعات ملکول زیادی برای شناسایی گونه های بابزیا و کنه های ناقل آن در نقاط مختلف ایران انجام شده است. هدف این مطالعه مروری، فراهم نمودن اطلاعات مفید در باره تاریخچه، مشخصات، انتشار جغرافیایی و فراوانی گونه ها بابزیا و کنه های ناقل در حیوانات اهلی ایران می باشد.

واژگان کلیدی

بابزیا، کنه، حیوانات، ایران

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ارزیابی شیوع سروارهای سالمونلا در سگ های خانگی ظاهرا سالم در تهران، ایران

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چکیده

سالمونلوز به عنوان یک بیماری مشترک بین انسان و دام در نظر گرفته می شود. از آنجایی که اخیراً مراقبت از حیوانات خانگی رایج شده است، امکان انتقال بیماری از طریق تماس دهان و مدفوع اجتناب ناپذیر است. از سوی دیگر، مصرف بیش از حد آنتی بیوتیک های انسانی برای درمان دام باعث ایجاد مقاومت آنتی بیوتیکی و ظهور سروتیپ های جدید سالمونلا شده است. این مطالعه با هدف بررسی شیوع باکتری ها و مقاومت آنتی بیوتیکی آنها برای انتخاب آنتی بیوتیک مناسب برای کنترل بیماری انجام شد. در این مطالعه حضور سروارهای سالمونلا در ۲۵۶ نمونه مدفوع سگ خانگی با غنی سازی و استفاده از محیط کشت انتخابی بررسی شد. سپس وجود ژن های حدت و ژن های مقاومت آنتی بیوتیکی علاوه بر مقاومت فنوتیپی ضد میکروبی مورد بررسی قرار گرفت. از مجموع ۲۵۶ نمونه مدفوع، ۲۱ نمونه (۲/۸ ٪) از سگ‌های خانگی سالمونلا مثبت بودند که شامل *S. Senftenberg* و *S. Infantis*, *S. Enteritidis*, *S. Typhimurium* می شدند. بر اساس یافته‌های ما، همه سروارها دارای ژن‌های حدت *invA, invF, sitC, fimA* and *S. Typhimurium* مقاوم به آمپی‌سیلین (۱۰۰ ٪)، تتراسایکلین (۵۰ ٪)، اکسی تتراسایکلین (۷۵٪)، فلورفنیکول (۵۰٪) و لینکوسکتین (۱۰۰٪) بودند. درحالی که *S. enteriti-* *S. senftenberg* و *dis*, *S. infantis*, به آمپی سیلین، آمیکاسین، جنتامایسین و سیپروفلوکساسین حساس بودند. *S. Infantis* نیز به تمام آنتی بیوتیک ها حساس بود. یافته‌های ما نشان می‌دهد که سگ‌های خانگی منابع بالقوه سویه‌های سالمونلا هستند که طیف وسیعی از ژن‌های مقاومت و حدت را حمل می‌کنند. بنابراین، سگ های خانگی سالم می توانند نقش مهمی در سالمونلوز انسانی داشته باشند.

واژگان کلیدی

ژن مقاومت آنتی بیوتیکی، سگ خانگی، سالمونلا، ژن حدت

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تعامل کیسپپتین مرکزی با سیستم های ملانوکورتین، گابا، کورتیکوتروپین و نوروپپتید Y بر دریافت غذا در جوجه‌ها

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چکیده

کیسپپتین یک جزء کلیدی تولید مثل است که می تواند مستقیماً بر دریافت غذا در پستانداران تأثیر بگذارد. شواهدی وجود دارد که نشان می دهد ملانوکورتین، گابا، کورتیکوتروپین و نوروپپتید Y (NPY)، نقش واسطه ای در پاداش دارند. با این حال، چگونگی تعامل این مواد با مصرف غذای ناشی از کیسپپتین در پرندگان، هنوز مشخص نیست. بر این اساس، در این مطالعه، در مجموع ۱۰ آزمایش برای بررسی تأثیر متقابل کیسپپتین و این سیستم‌ها برای کنترل مصرف غذا در جوجه‌های نوزاد انجام شد. در آزمایش اول، جوجه ها با سالین و متاستین (Kisspeptin ۰/۵ ، ۰/۲۵ ، و ۱ نانومول) بصورت داخل بطن مغزی (ICV) تزریق شدند. در آزمایش دوم، سالین، متاستین (۱ نانومول)، BIBP-3226 (آنتاگونیست گیرنده NPY1، 1.25 نانومول)، و تزریق همزمان متاستین + BIBP-3226 تزریق شد. آزمایش‌های ۱۰-۳ مشابه آزمایش ۱ بودند، با این تفاوت که جوجه‌ها BIIE 0246 (آنتاگونیست گیرنده NPY2، 1.25 نانومول)، CGP71683A (آنتاگونیست گیرنده NPY5، ۵۰ میکروگرم)، پیکروتوکسین (آنتاگونیست گیرنده GABAA، 1.25 نانومول)، CGP54626 (آنتاگونیست گیرنده GABAB، ۲۱ میکروگرم)، Astressin-B (آنتاگونیست گیرنده CRF1 / CRF2، 30 میکروگرم)، Astressin2-B (آنتاگونیست گیرنده CRF2، 30 میکروگرم)، SHU9119 (آنتاگونیست گیرنده MC3 / MC4 ملاتونین، ۰/۵ نانومول) و MCL0020 (آنتاگونیست گیرنده / MC۳ MC۴ ملاتونین، ۰/۵ نانومول) را بجای BIBP-3226 دریافت کردند. سپس مصرف غذا تا ۱۲۰ دقیقه پس از تزریق ارزیابی شد. باتوجه به نتایج بدست آمده متاستین (۰/۲۵؛۰/۵ و ۱ نانومول) موجب افزایش معنی دار مصرف غذا بصورت وابسته به دوز شد ($p \leq ۰.۰۵$). هر چند BIBP-3226 و پیکروتوکسین هیپرفاژی ناشی از متاستین را در جوجه های نوزاد مهار کردند ($p \leq ۰.۰۵$). در حالیکه BIIE 0246 ، CGP71683A، CGP54626، astressin-B، astressin2-B، SHU9119 و MCL0020 اثری نداشتند($p > ۰.۰۵$) . این نتایج نشان داد که اثر کیسپپتین بر دریافت غذا ممکن است توسط گیرنده‌های NPY1 و GABAA در جوجه‌های نوزاد واسطه گری شود.

واژگان کلیدی

کورتیکوتروپین، اخذ غذا، مرغ تخمگذار، NPY، کیسپپتین، گابا، ملاتونین

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عصاره هیدروالکلی سه گونه گیاه Artemisia درد پالپ دندان و اختلال رفتار تغذیه ای ناشی از درد را در موش های صحرایی کاهش می دهد.

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چکیده

این مطالعه پتانسیل درمانی عصاره هیدروالکلی سه گونه از گیاه دورمنه (Artemisia) بر درد پالپ دندان و تغییرات ناشی از درد بر رفتار تغذیه ای را در موش های صحرایی نر بالغ مورد بررسی قرار داد. حیوانات در پنج گروه (n=۶) شامل: گروه حلال، گروه کپسایسین و گروه های کپسایسین درمان شده با عصاره های *A.persica*، *A. sieberi* و *A.biennis* تقسیم بندی شدند. درد پالپ با تزریق داخل دندان کپسایسین(۱۰۰ µg) ایجاد شد. ده دقیقه قبل از تزریق کپسایسین عصاره های هیدروالکلی(۲۰۰ mg/kg) به صورت داخل صفاقی تزریق گردید. رفتار درد برای مدت ۴۰ دقیقه ثبت شد. نتایج نشان داد ، درمان با هر سه عصاره، درد پالپ دندان القا شده با کپسایسین را کاهش می دهد. علاوه براین، تزریق کپسایسین تعداد ورود به ناحیه حاوی آب و غذا دستگاه ارزیابی رفتار و مدت زمان و میزان غذا خوردن را کاهش داد. اثرات نامطلوب کپسایسین بر کاهش مدت و تعداد دوره های مصرف غذا در گروه درمان شده با A.persica کاهش یافت. علاوه براین، همه عصاره های استفاده شده اثرات مهاری کپسایسین بر مصرف آب و غذا را کاهش دادند. در مجموع ، مطالعه حاضر نشان داد عصاره های گیاه Artemisia در کاهش درد پالپ دندان و اختلال در رفتار تغذیه ای ناشی از درد در موش های صحرایی موثر است.

واژگان کلیدی

موش های صحرایی ،Artemisia ،درد پالپ، کپسایسین، مصرف غذا

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اثر ضدتشنج آرتمیزینین بر تشنج های ناشی از پنتیلن تترازول در هنگام چرخه قاعدگی و برهمکنش با گابا در موش

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چکیده

تشنج قاعدگی یا تشدید تشنج در زمان های ویژه ای از چرخه قاعدگی می تواند ۷۰-۱۰ درصد از زنان دچار تشنج را دربرگیرد. آرتمیزینین ترکیبی گیاهی با کاربردهای گسترده درمانی سنتی و مدرن است. بر اساس برهمکنش با سامانه گابائرژیک، هدف این پژوهش، بررسی اثر ضدتشنج آرتمیزینین در زمان چرخه قاعدگی است. ۳۶۰ موش ماده برنا در ۱۰ گروه: کنترل، آرتمیزینین(75& 100 mg/kg)، بیکوکولین(۲mg/kg)، بیکوکولین+ آرتمیزینین(100&75 mg/kg)، ساکلوفن(۲ mg/kg)، ساکلوفن+ آرتمیزینین(100& 75 mg/kg) و حلال(اتانول ۱۰ ml/kg) هرکدام با ۴ زیرگروه پرواستروس؛ استروس، مت استروس و دی استروس استفاده شد. پس از ۱ هفته سازگاری، همزمان سازی و شناسائی مرحله قاعدگی انجام شد. ۳۰ دقیقه پس از تزریق آرتمیزینین و اتانول، تشنج حاد با تزریق درون صفاقی (۸۰ PTZ mg/kg) برانگیخته شد. سپس میزان مرگ، مدت تشنج، زمان آغاز تشنج های تونیک- کلونیک و میوکلونیک برای۳۰ دقیقه ثبت گردید. داده ها به صورت میانگین ± انحراف معیار ثبت و با آزمون های آنوای یک سویه و توکی کرامر بررسی شدند($p < ۰.۰۵$). آرتمیزینین سبب کاهش معنی دار رخداد، مدت و مرگ به موازات افزایش زمان آغاز تشنج های تونیک- کلونیک و میوکلونیک شد که در گام لوتئال از فولیکولار برجسته تر بود. تجویز همزمان بیکوکولین سبب مهار معنی داری اثرضد تشنج آرتمیزینین گردید، درحالیکه ساکلوفن چنین برهمکنش مهاری نداشت. چنین می نماید که افزایش دگرگشته های نورواستروئید و گیرنده های گابا، هیپرپلاریزه شدن عصبی به دنبال برهمکنش با گیرنده GABAA، ویژگی های ضدآماس و ضداکسیدان که سبب کاهش آماس و برانگیختگی عصبی می شوند می تواند در کارکرد ضد تشنج آرتمیزینین اثرگذار باشد.

واژگان کلیدی

آرتمیزینین، تشنج قاعدگی، چرخه قاعدگی؛ گابا

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Author Index

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GUIDE FOR AUTHORS

IRANIAN JOURNAL OF VETERINARY SCIENCE AND TECHNOLOGY

Guide for authors

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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:

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

The following information must be provided in the section:

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

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

References for the above example:

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

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

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

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

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

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

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

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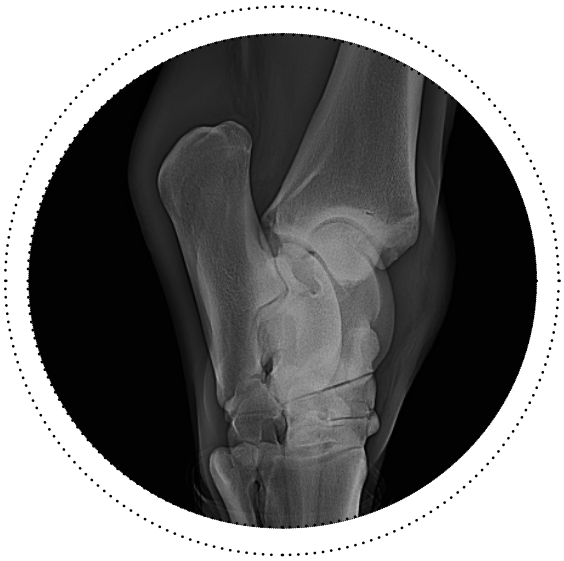

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