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Iranian Journal of Veterinary Science and Technology (IJVST) is a peer-reviewed and multi-disciplinary journal that supports important research advances in veterinary medicine and subject areas relevant to veterinary medicine including anatomy, physiology, pharmacology, bacteriology, biochemistry, biotechnology, food hygiene, public health, immunology, molecular biology, parasitology, pathology, virology, etc. Contributions related to clinical sciences including large and small animal medicine, poultry disease, diseases of equine species and aquaculture are welcomed. Articles can comprise research in basic sciences, as well as applied veterinary findings and experimental studies and their impact on diagnosis, treatment and prevention of diseases.

ON THE COVER

Calotropis procera (giant milkweed) is a cardiototoxic plant which is found in Iran. This plant contains a number of cardiac glycosides including calotropin and calotoxin. Ingestion of different parts of C. calotropis by domestic animals can lead to acute cardiotoxicity with high lethality, manifested by cardiac dysrhythmias and multi-focal coagulation necrosis of cardiac fibers. Accidental contact of C. calotropis latex into the eye also causes severe kerato-conjunctivitis. The original photo was taken in Doha, Qatar, June 27, 2013, and is courtesy of Alexey Sergeev (http://www.asergeev.com). Illustration: Taraneh Ebnalnassir. See page 1.
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Cardiotoxicity of Plants in Iran: a Review

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Keywords
Oleander poisoning, yew poisoning, cardiac glycosides, taxines, cardiac arrhythmia

Abstract
In this review an attempt has been made to review the cardiotoxic effects of poisonous plants which are found in Iran. Among various plant species growing in different regions of Iran, a large number of plants contain chemical compounds which are toxic to animals and humans. Among those poisonous plants, cardiotoxic plants are important due to the acute nature of their toxicity and frequent lethal livestock and human intoxications. Cardiotoxic plants of Apocynaceae family, Nerium oleander, N. indicum and Thevetia peruviana contain cardiac glycosides including oleandrin, oleanandroside and thevetin A-C. Plants of Taxaceae family, Taxus baccata and T. brevifolia contain taxine alkaloids, including taxine A and B. The toxic effects of cardiac glycosides are primarily attributed to inhibition of plasmalemmal Na+/K+-ATPase which results into the accumulation of intracellular Ca²⁺ and, depending to its severity, inotropic or arrhythmic effects are seen. Taxine B, the prominent alkaloid in Taxus spp. block sodium and calcium channels preferentially in cardiac myocytes, thus causing conduction abnormalities. Various cardiac arrhythmias in acute cases of poisoning with aforementioned plants result in acute heart failure and death. Post mortem findings are non-diagnostic and toxicological analysis of gastrointestinal tract content or body fluids is used for detection of cardiac glycosides and taxines.

Abbreviations
CGs: cardiac glycosides
Na+/K+-ATPase: sodium/potassium adenosine triphosphatase
SR: sarcoplasmic reticulum
RyR2: ryanodine receptor 2
AV: atrioventricular
ECG: electrocardiography
GI tract: gastrointestinal tract
TLC: thin layer chromatography
LC-MS/MS: liquid chromatography tandem mass spectrometry

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Introduction

Different phytogeographic regions in Iranian plateau have caused massive genetic flow in this area resulting to the generation of a variety of plant species. Total Taxa in Iran are about 8,000 which include about 6417 species, 611 subspecies, 465 varieties, and 83 hybrids [1], many of which have the potential to be toxic to animals and humans [2]. Economic and financial losses caused by poisonous plants have been significant over the years and require more attention and research efforts. These losses result from death of livestock, weight loss, decreased production, birth defects, management alternations and medical costs [2, 3, 4]. Many plant species are also poisonous to humans and intentional or accidental exposure leading to illness and fatality are occasionally seen, particularly in rural areas [5, 6]. Among the poisonous plants, cardiotoxic plants are important due to the acute nature of their toxicity and frequent lethal intoxications in livestock and human [2, 7]. The present review article describes various features and aspects of poisoning with the most important cardiotoxic plants in Iran.

Cardiac glycoside-containing plants

Several species of plants found in Iran contain cardiac glycosides (CGs) (Table 1), however, the toxic effects on animal or human health differ widely among these plant species. Three species of Apocynaceae (Dogbane family), Nerium oleander, N. indicum and Thevetia peruviana contain CGs, and are known as extremely toxic plants, causing lethal intoxication in animals and humans. These species are perennial evergreen ornamental plants and are planted as large flowering shrub or small ornamental trees in gardens, parks and road sides. Nerium species, particularly N. oleander are wildly grown as ornamental plants in most parts of Iran, while cultivation of T. peruviana, yellow oleander, is limited to the southern lowland regions [2, 8].

Botanical description

Nerium species vary in size from shrubs to small trees, 2-4 m in height, with erect stems and branches. Leaves are elongated, dark green to grey green in color, leathery, about 1 cm wide and 8 to 22 cm long with a prominent mid rib. Flowers are white to pink to deep red, with 5 spreading petals (Figure 1). The fruit is a narrow pod and contains many silky-haired seeds. The sap is thick, gummy and clear [7]. The color and the type of flowers are used to distinguish different varieties; the presence of variegated leaves and the growth habits can also be differentiating criteria [9].

![Figure 1. Nerium oleander, red and white flowers. Photograph by M.R. Aslani.](image1)

![Figure 2. Thevetia peruviana, light orange and yellow flowers and fruits. Photograph by M.R. Aslani.](image2)
T. peruviana is mostly seen as a small ornamental tree, with diffusely branched and dense crown and 2.5 - 2.3 m height. The leaves are dark green, linear, spirally arranged, glossy and about 13-15 cm in length. Flowers are in small clusters at the tip of twigs, funnel-shaped with 5 petal lobs and yellow to dull orange color (Figure 2). The fruits which are somewhat globular, slightly fleshy, green in color with a diameter of 4-5 cm, become black on ripening and contain 2 seeds. All parts of the plant contain the milky juice [7].

Toxic constituents

Cardioactive steroids or CGs are the most noticeable naturally-occurring compounds identified in Nerium species and T. peruviana (Table 2). All parts of these plants whether fresh, dried or boiled contain cardiac glycosides of cardenolide class and are toxic. About 30 types of CGs from N. oleander and 15-18 types of CGs from T. peruviana have been isolated which were unevenly distributed throughout the plant, some being common in all parts of the plants [10, 11]. These glycosides are structurally derived from the tetracyclic 10,3-dimethylcyclopentanoperhydrophenanterene ring system with a 5 or 6 membered unsaturated lactone ring attached at the 17-position and sugar molecules which usually attached at the 3-position (Figure 3). The sugar molecules influence pharmacokinetic characteristics including water solubility, cell penetrability and duration of action, while pharmacological and toxicological properties of the glycosides reside principally in the unsaturated lactone ring at C-17 [12]. Acid hydrolysis leads to a cleavage of the glycoside into aglycones and sugar residues, which strongly decreases the biological activity of the glycoside, while more rigid conditions (alkaline hydrolysis) lead to cleavage of the lactone ring and to a total loss of its activity [13].

The most prominent CGs of Nerium species are oleandrin, nerioside and oleandroside. Oleandrin is the most studied CGs in these plants and it is a complex molecule that is very similar to digoxin and digitoxin, cardiac glycosides originated from Digitalis species which are used for treatment of congestive heart failure. It has been demonstrated that T. peruviana seeds contain a mixture of at least six CGs in-

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<th>Nerium species</th>
<th>T. peruviana</th>
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<tr>
<td>Oleandrin</td>
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<td>Oleandrosid</td>
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<td>Digitoxigenin</td>
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Figure 3. General structure of cardenoloids.

Figure 4. Chemical structure of oleandrin (top) and Thevetin A (bottom).
Cardiotoxicity of Plants in Iran

including thevetin A, B and C, and neriifolin [14, 17] (Figure 4). It has been reported that in *N. oleander* the leaves contain the highest oleandrin contents and roots and seeds typically containing the highest contents of CGs. On the other hand, a significant variability of oleandrin has been indicated depending on varieties, a parameter which is related to the genetic variability between the wild shrub and different cultivars which differentiated by color and morphology. The plant height and some habitat conditions such as rate of light exposure or the type of soil are also factors that may influence the content of the oleandrin in the plant [18].

**Plant toxicity**

Oleander leaves have a bitter taste and it is said that is not palatable for livestock, and these animals seldom eat the plant voluntarily. Mastication of oleander leaves releases saponins that are surface active agents, and cause a burning sensation upon contact with tissues of the oral cavity, thus, rendering the leaves unpalatable [20]. However, extremely hungry cattle, sheep, and goats have been poisoned when grazing a strange pasture containing little forage but oleander. Apparently dry leaves more readily accepted by livestock, particularly when prunings are mixed with grass clippings or incorporated in baled hay, and the bitter taste is disguised [21]. In fact, dried oleander leaves mixed with forage is the common cause of oleander poisoning in herbivorous animals.

Various factors affects the amount of toxic glycosides within oleanders, therefore, it is difficult to determine a lethal dose for these plants poisoning in animals or humans. In this regards, Rezakhani and Maham reported the lethal dose of dry oleander leaves as 30 and 50 mg/kg body weight for donkeys and cattle, respectively [22], while other experimental studies have suggested the lethal dose of dry oleander leaves as 110 mg/kg body weight for calves, sheep and goats [23, 24, 25].

The determination of lethal dose of oleanders poisoning for humans is more difficult because of few published case reports that contain data sufficient to assess specific quantities of ingested doses. Although one leaf has been considered potentially lethal, ingestion of larger amounts is probably necessary to produce serious toxicity [13]. Ingestion of 5-15 *N. oleander* leaves or 8-10 *T. peruviana* seeds has resulted in fatal poisoning in adults [7]. Inhalation of smoke from burning oleander leaves and twigs, use of the sticks to roast meat or marshmallows, drinking of water in which the flowers or leaves have been soaked, partaking of a soup that was stirred with an oleander branch, and the ingestion of sap or honey produced from oleanders has resulted in poisonings of humans [26, 27]. Cutaneous, oral and trans-rectal use of some oleander preparations as indigenous herbal medicine has also been resulted in human intoxication [28, 29].

Little is known about toxicokinetics of cardenolides during oleander intoxication in animals. There are some evidence in experimentally induced oleander intoxications that the glycosides rapidly absorbed from gastrointestinal tract so that massive doses of leaves may kill an animal within 1 hour [30]. It has been suggested that after yellow oleander seed ingestion and absorption of glycosides into the systemic circulation, oleander glycosides are re-secreted into the gut lumen and disrupting the enterohepatic cycling will increase elimination of toxic glycosides [31]. In murine model, a considerable amount of oleandrin (approximately 60%) is excreted via biliary system through feces, whereas urinary excretion is not considered a major route of elimination (>10%). It has also been suggested that components within the oleander may enhance the transport of oleandrin across the blood brain barrier [32].

The CGs such as digoxin in humans are excreted from the udder and its concentration in the breast milk is approximately equal to the unbound plasma digoxin concentration [33]. Cardiac arrhythmias has been observed in suckling calves of oleander poisoned cattle, suggesting the passage of the oleander glycoside through the milk [34, 35].

**Risk factors**

There is substantial variation in the sensitivity to oleander poisoning among animal species. Generally, ruminants are less sensitive than monogastric animals. In ruminants solubilized CGs in the rumen can lead to noticeable loss of action as a result of hydrolytic splitting of the digitoxin. Dogs, cats, monkeys, and humans are relatively sensitive to the effects of oleander-derived cardenolides, whereas rodents and avian species are relatively insensitive [13]. The similarity in sensitivity to CGs between humans and dogs is probably due to the very close structural similarity of the Na+/K+ -ATPases found in these two species. In contrast, it has been shown that the rat cardiac Na+/K+ -ATPase exhibits a low affinity towards cardiac glycosides [20]. The time until the onset of symptoms, severity and outcome of intoxication all depend on parts and amount of the ingested plant. On the other hand, frequent vomiting in humans and animals such as dogs is a protective mechanism, helping to survive the intoxicated cases [2].

Hypomagnesemia can worsen CGs toxicity and
predispose to dangerous arrhythmias, therefore, monitoring of serum magnesium level and its correction to normal may be advisable [36]. In this regards, administration of magnesium has often been found to abolish arrhythmias caused by CGs [37]. Inversely, intravenous administration of calcium containing fluids is dangerous in cases of CGs poisoning [11]. Since the intracellular level of Ca$^{2+}$ is elevated in CGs intoxication, administration of calcium may worsen cardiac arrhythmia and animal data have reported increased toxicity including death, which may relate to sustained cardiac contraction, also known as 'stone heart' [36, 38].

**Pathophysiology**

The Mg$^{2+}$ dependent Na$^+$/K$^+$-ATPase in cardiac myocytes that supplies energy for the active pumping of Na$^+$ outward and K$^+$ inward the cell membrane, is believed to be the cellular receptor for cardiac glycosides [39]. All cardiac glycosides bind specifically to and inhibit the sarcolemmal Na$^+$/K$^+$-ATPase. The inhibition of Na$^+$/K$^+$-ATPase results in the accumulation of the Na$^+$ within the cardiac myocytes. This local accumulation of Na$^+$, in turn, causes an increase in Ca$^{2+}$ concentrations as the Na$^+$/Ca$^{2+}$ exchanger promotes Ca$^{2+}$ influx over efflux. Elevation of cellular Ca$^{2+}$ concentration is responsible for the inotropic action of cardiac glycosides such as digoxin.

The toxic effects (ie, arrhythmias) occur when the cytoplasmic Ca$^{2+}$ increases to concentrations exceeding the storage capacity of the sarcoplasmic reticulum. As a consequence of this internal Ca$^{2+}$ overload, spontaneous release of Ca$^{2+}$ (Ca$^{2+}$ waves) from the sarcoplasmic reticulum (SR) through ryanodine receptor 2 (RyR2) channels occur as a process known as store overload-induced calcium release. In addition, high internal concentrations of Ca$^{2+}$ activate a depolarizing (inward) current corresponding to the forward mode of the electrogenic Na$^+$/Ca$^{2+}$ exchanger (3Na/2Ca). This current generates delayed after-depolarizations that give rise to extra-systoles and polymorphic ventricular tachyarrhythmias due to triggered activity [40, 41, 42].

Furthermore, recent studies have suggested an alternative mechanism for pro-arrhythmic alterations in myocyte Ca$^{2+}$ handling caused by CGs involving the generation of reactive oxygen species (ROS). The ROS release from mitochondria upon exposure to CGs. CG-induced ROS activate Ca$^{2+}$/calmodulin-dependent protein kinase II resulting in increase phosphorylation of RyR2 and pro-arrhythmic Ca$^{2+}$ waves [43].

From an electrophysiological perspective, the negative chronotropic activity of CGs is largely attributed to the increased vagal tone, which decreases the rate of sinoatrial node depolarization and increased refractory period of the atrioventricular node (AV). The result is a reduction in sinoatrial and AV conduction [44].

**Clinical findings**

Many animals exposed to oleanders are often found dead. Clinical signs in experimental cases develop about 1.5 hours after ingestion of the oleander leaves. In accidental cases it may be delayed depending on the type of plant material ingested and the level of mastication of that materials. The signs may persist up to 7 days in nonfatal cases. Pollakiuria is the first sign that appears after plant exposure, concurrently, bradycardia with strength beats are noticeable in heart auscultation that may be transient in some cases. Then, abdominal pain is developed and progressed in severity and manifested by restlessness, dental grinding and grating, forelimb pawing the ground, kicking at the belly, swishing of the tail, frequent lying and getting up, humped posture, flank-watching, frequent defecation and tenesmus. Initial bradycardia followed by heart blocks, ventricular premature beats and ventricular tachycardia and, ventricular fibrillation is the end stage of the poisoning.

Affected animal also shows some degrees of salivation, frothing of the mouth, tremor, depression and lethargy, ruminal atony with moderate bloat and diarrhea. Blood is often appeared as clots or streaks in feces in later stages. In some cases heart beats may be audible without the stethoscope. Rectal temperature may be normal or slightly subnormal. Lethal dose of oleander leads to death in most cases of ruminants within 12 hours. Death occurs following prostration and severe convulsive movements, general spasm and bellowing [2, 24, 25, 35].

Horses may develop clinical signs of toxicosis within a few hours after ingestion of oleander leaves [30]. However, sudden death is the most common finding attributed to oleander poisoning in equines. The most commonly reported signs are nonspecific initially and may include lethargy and depression with either profuse watery diarrhea, decreased intestinal motility or ileus. Other signs may include muscle tremors, profuse sweating, ataxia, weakness, colic, elevation of capillary refill time and seizures along with a variable degree of bradycardia and heart blocks. In later stages, hypothermia, recumbency, ventricular tachyarrhythmias, and dyspnea are seen. Death usually occurs within 12–36 hours after the development of signs due to cardiac dysfunction [45, 46].
The clinical signs of oleander poisoning in dogs occur very fast, within an hour, after the ingestion of the leaves. The first and most common clinical sign is vomiting. Other clinical signs of oleander poisoning in dogs may include loss of appetite, salivation, nausea, apathy, congestion of conjunctiva, dehydration, abdominal pain, tremor, diarrhea and tenesmus [11, 47]. Signs of oleander cardiotoxicity in dog is manifested as variety of dysrhythmias including sinus bradycardia, 2nd degree atioventricular block, ventricular premature complexes, sinus tachycardia and ventricular tachycardia [11].

Oleander poisoning has been described in camelids including one humped camels, llamas and alpacas. The toxicity in one humped camel is manifested by dullness, vomiting, shivering, yawning, ataxia, excitement or convulsion followed by diarrhea and coma in severely affected cases [48]. Clinical findings of oleander poisoning including anorexia, lethargy, decreased to absent auscultable gastrointestinal tract motility, diarrhea, bradycardia, sinus arrhythmia, 2nd degree A-V block, azotemia and hyperglycemia have been recorded in new world camelids [49].

Ingestion of any part of oleander plants can result in a variety of signs similar to digoxin poisoning in humans. The onset and severity of intoxication depends on parts and amount of ingested plant, preparations and individual susceptibility [7, 13]. Clinical signs appear within a few hours following ingestion of plants. The GI signs includes excessive vomiting, nausea, abdominal pain, diarrhea, dry mouth, burning of the mouth and paresthesias of the tongue. As other species, the most common serious complication of oleander poisoning in humans is disruption of cardiac conduction featured as sinus bradycardia, SA and AV blocks, atrial fibrillation which may persist 3-6 days. Life-threatening ventricular tachyarrhythmias and fibrillation, cardiovascular collapse or cardiogenic shock may occur in severe oleander intoxication [7, 13, 17]. Neurological signs of oleander plants intoxication seen in humans include blurred vision, visual disturbances such as halos, weakness, tremor confusion, dizziness, headache, fainting, depression, drowsiness and lethargy [7].

**Electrocardiographic abnormalities**

ECG abnormalities in cases of oleander poisoning include sinus bradycardia, prolonged P-R interval, sino-atrial block, 1st and 2nd degree A-V blocks, A-V dissociation and sinus tachycardia. In acute cases of oleander poisoning those findings are transient, but more dangerous cardiac arrhythmias including unifocal and multifocal ventricular premature contractions (Figure 5), ventricular tachycardia, depression or elevation of S-T segment and ventricular fibrillation in the end stage are seen [22, 23, 24, 25].

**Clinical pathology and toxicological analysis**

Elevation of serum glucose, BUN and creatinine have been reported in horses and new world camelids poisoned with oleanders [45, 49]. Two-dimensional thin layer chromatography (TLC) has been used for identification of oleandrin in tissue samples and GI contents of livestock poisoned with oleander [50]. The high performance TLC has also been described as a simple, rapid and efficient method for the isolation and residual determination of oleandrin from oleander plant and autopsy samples [19].

For detection of oleander cardiac glycosides in blood fluorescence polarization immunoassay has widely been used in human intoxicated cases [7]. A
rapid liquid chromatography tandem mass spectrometry (LC-MS/MS) method, using a triple-quadrupole/linear ion trap mass spectrometer, has been developed for the quantitative determination of oleandrin in serum, urine, and tissue samples and suggested to be the method of choice for toxicological investigations of oleander poisoning [51].

Pathology

Postmortem lesions of animals died due to intoxication with oleanders is not pathognomonic. Findings at gross are often associated to acute heart failure as fluids in body cavities, congestion and hemorrhages of the subdermal tissues, epicardium and endocardium, liver, kidneys and gastrointestinal tract. Lungs are also congested and edematous [24, 25]. Fragments of the oleander leaves may be recognized in forestomachs/stomach contents [52]. Histologically, evidence of myocardial degeneration and necrosis associated with hemorrhage and infiltration of mononuclear inflammatory cells in the heart, fatty changes, focal degeneration and necrosis of hepatocytes, congestion and tubular epithelial necrosis in the kidneys and perivascular and perineuronal edema and hemorrhages in cerebrum have been reported in oleander poisoning in animals [24, 25].

Cardiotoxic alkaloid-containing plants

Plant description

Yews (Taxus spp.) are popular evergreen trees or shrubs with high longevity and slow growth, belonging to the family of Taxaceae and used as ornamental landscaping plants in many parts of the world. Two species of genus Taxus, T. baccata and T. brevifolia are found in Iran of which only T. baccata is native. T. baccata grows in northern parts of Iran in 900-1800 meters above sea level and because of reddish-brown bark, is known indigenously as Sorkhdar [53, 53]. The height of the plant reaches up to 28m. Leaves are small needle-like in opposite pairs, waxy, linear, dark green, 1-3cm long and 2-3mm wide with midrib prominent on both side and cane live up to 8 years. Branches are long and not whorled (Figure 6). Yew produces soft, bright berry like structure fruit called an aril, contains a single seed and has sweet taste which matures within 6 to 9 months [55].

Toxic constituents

Yews contain at least 10 taxine alkaloids with taxine A and taxine B the most widely recognized as cardiotoxins [52]. Of the two, taxine B constitutes about 30% (approx. 1.2% of dry weight in leaves) and taxine A only constitutes approximately 1.3% of total alkaloids. Taxine B alkaloids have much more cardiotoxicity than taxine A [56, 57]. Taxines are a mixture of alkaloids formed from nitrogen-free polyhydroxyl diterpenes esterified with β-dimethylamino-β-phenylpropionic acid and acetic acid. Taxines are particularly instable in neutral and alkaline environment and their toxicity decreases over time. These compounds are only slightly soluble in water but are readily soluble in alcohols, chloroform, dilute acids, low ketones and low esters [57].
**Plant toxicity and risk factors**

Fresh or dried yew plants are highly toxic throughout the year and have been implicated in poisonings and fatalities in different animals as well as humans. All parts of the plant except the scarlet aril (berry) surrounding the seed contains toxic alkaloid. The seeds within the aril are extremely toxic [57]. However, the seeds are not toxic if swallowed in whole because the seed coat resist digestive enzymes. The maximal concentration of taxines in the foliage occurs during the winter season and the mature leaves are more toxic than the new ones [58]. It has also been reported that taxines are relatively abundant in *T. baccata* while minimal amounts are found in *T. brevifolia* [59, 60]. Poisoning in animals is often accidental and is frequently occur following exposure to discarded yew foliage of trimmings near their pasture or when livestock have access to landscape plot [61].

Yews are toxic to all animals to varying degrees. Horse are the most sensitive animals [57]. Most cases of poisonings have involved cattle and horses, however is has been reported in variety of animal species including canine and birds [61]. Some species of *Cervidae* including white-tailed deer (*Odocoileus virginianus*) and roe deer (*Capreolus capreolus*) eat yew over time without any adverse effects, due to elevation of ruminal and hepatic adaptation to detoxify taxines [57]. The minimal toxic dose of yew leaves for horses is 0.2-0.4, for cows 2, for sheep 2.5, for goats 12, for chicken 16.5 g/kg body weight [52, 57].

The incidence of yew poisoning may be higher in the winter when the other green plant materials are not available for animals to eat, the alkaloid content of the plant is highest and relative palatability of the leaves increases. Animals with hepatic disease may also be at higher risk of poisoning because the taxines are metabolized in the liver by conjugation and excreted in the bile and urine [60, 62, 63].

**Pathophysiology**

The taxine alkaloids are absorbed through the digestive tract rapidly, and the signs of poisoning are seen after 30-90 minutes [64]. Because of instability, purified taxine A and B are not available and crude extract of yew is used for study on the mechanism of action of taxines. From the experimental studies it has been concluded that the mechanism of action of taxines is primarily based on their sodium and calcium channel antagonistic properties, preferentially in cardiac myocytes, thus causing conduction abnormalities [57]. The inhibition of those channels results in decreased intracellular calcium within the myocardial cells which cause slowing of conduction, decreased atrial and ventricular contractility, bradycardia, arrhythmia, diastolic cardiac arrest and decreased cardiac output. Decreased cardiac output coupled with vasodilative effect of taxine result in profound hypotension [65]. On the other hand, interference of taxine alkaloids with potassium ion channels leading to hyperkalemia and absence of P wave and long duration of QRS complex in ECG has also been speculated [66].

**Clinical findings**

In most cases of acute poisoning, animals are often found dead or die within 3-4 hours after ingestion of the plant materials with no evidence of struggle. Subacute cases of yew intoxication in cattle which are seen infrequently is characterized by ataxia, bradycardia, anxiety or depression, dyspnea, muscle tremors, weakness, hypothermia, rumen hypotony which progressed to atony in later stages, sternal or lateral recumbency, and collapse and death without any struggling [52, 57, 61, 67]. Abortion has been reported in late-term pregnant cattle within 4 days of yew ingestion [61]. In cases of yew poisoning in horses, symptoms of intoxication are less or more similar to those observed in ruminants. The onset of signs may occur within an hour of yew ingestion and the course of clinical intoxication may last 15 to 45 minutes. Clinical signs include incoordination, weak pulse, nervousness, dyspnea, respiratory grunt, trembling of legs, decreased lip and tail tone, collapse and recumbency [58, 61]. The clinical signs in a nonfatal case of yew ingestion in a dog included mydriasis, tetanic seizures, increased aggressiveness and gastroenteritis lasting for one week [68].

Yew leaves and seeds have been used in humans for homicide, suicide or folk medicine. Clinical signs of poisoning in those cases may include nausea, vomiting, dizziness, diffuse abdominal pain, pupil dilation, muscle weakness, seizures, tachycardia (initially), cardiac arrest, respiratory paralysis and death. These signs can proceed to bradycardia, bradypnea, diastolic cardiac standstill or death [57, 69].

**Electrocardiographic abnormalities**

In experimentally intoxicated sheep by yew leaves ECG abnormalities include sinus tachycardia, sinus bradycardia, ventricular premature beats, multifocal ventricular tachycardia, idioventricular and idiojunctional rhythm with arrest and silent atria, atrial fibrillation, atrial tachycardia, S-T segment slurring, QRS and T widening, AV dissociation, S-T segment...
depression, episodes of cardiac pacemaker arrest, R on T phenomenon, inverted T and ventricular fibrillation [67, 70, 71].

**Clinical pathology and toxicological analysis**

Serum chemistry changes are limited in acute cases of yew poisoning. Hyperkalemia may be seen in some cases [66, 67]. Chemical analysis of GI content including stomach/rumen content by GC/MS, LC/MS or TLC can be used to confirm the presence of taxine alkaloids [57].

**Pathology**

Generally, the postmortem evaluation is usually unremarkable, because sudden death occur in most cases of yew poisoning and gross or microscopic lesions are rarely observed. Yew leaves or fragments are found in GI content particularly in stomach or rumen content of most cases which can be considered as a basic for diagnosis [52, 58, 69] Some degrees of hemorrhage on the cardiac surface, pulmonary edema and myocardial necrosis have been observed in accidental yew poisoning in horse and calves [58, 62]. General vascular dilation, hepatic and splenic congestion; pulmonary edema, petechial hemorrhages on the epicardium and endocardium, multifocal nonsuppurative interstitial myocarditis with mild focal cardiac muscle cell degeneration and necrosis as well as hyperemia and focal hemorrhages, moderate to severe interstitial edema with inflammation of the sinoatrial node and the AV-node, the bundle branches and the His-bundle have also been identified in sheep experimentally intoxicated with yew leaves [67].

**Conflict of interest**

The author declares that there is no conflict of interest.

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Preparation and in vitro evaluation of chitosan-based films for the sustained delivery of enrofloxacin

Ali Rassouli, Sakineh Khanamani Falahatipour, Yalda Hosseinzadeh Ardakani, Hamid Akbari Javar, Katayoun Kiani, Taghi Zahraee Salehi

Keywords
Enrofloxacin, chitosan, β-glycerophosphate, sustained release, triple-layer film

Abstract
The implantable drug products are developed mainly to sustain the drug release. This study was conducted to formulate and evaluate cross-linked films of chitosan/β-glycerophosphate (β-GP) for the sustained delivery of enrofloxacin (ENR). Two types of formulations, single-layer (F1 and F2) and triple-layer (F3 and F4) films, were prepared. In vitro drug release, kinetic modelling, Fourier transform infrared spectroscopy (FTIR) spectra, morphological and microbiological studies were performed. Drug release from F1 and F2 continued up to 5 hours but from F3 and F4, it was extended over 96 and 168 hours, respectively. The cumulative drug release for F1, F2, F3 and F4 were 72.6, 70.1, 90.5 and 82.4%, respectively. The inhibition zones of bacterial growth by using positive controls and single layer films were significantly greater than those of triple-layer films (p < 0.05), indicating sustained drug release pattern of the multi-layer films. These findings suggest that the triple-layer chitosan/β-GP films could be effective to deliver ENR for a long period.

Abbreviations
ENR: Enrofloxacin;
FTIR: Fourier transform infrared spectroscopy;
β-GP: β-glycerophosphate

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Introduction

Novel drug delivery systems are going to be developed to optimize the therapeutic properties of drug products and make them to be safer, more effective, and reliable. Advantages of the implantable drug-delivery systems may include improved efficiency, reduced dosage, reduced side effects, on-spot delivery, relatively linear drug delivery for longer periods of time, and as a result, maintaining the plasma drug levels continuously in a therapeutic range. The major driving forces for the development of innovative veterinary sustained release products include the reductions in the duration of drug therapy, the frequency of drug dosing, and the imposed stress to the animals [1, 2].

Hydrogels are cross-linked, three dimensional networks of linear hydrophilic polymers capable of absorbing large amounts of water while remaining insoluble [3]. Hydrogels can be formulated in a variety of physical forms, including slabs, microparticles, nanoparticles, and films. When a drug is incorporated into a polymer solution, it becomes entrapped within polymer matrix upon its solidification, and the drug release occurs over time as the polymer degrades gradually in the body [4].

Chitosan is a natural cationic copolymer derived from chitin [5]. It is highly interesting for pharmaceutical applications because of its high solubilization capacity, lack of toxicity, biocompatibility, biodegradability, low cost, high mechanical strength and good film-forming ability [6]. Recently, the development of crosslinking methods has been attempted to improve structural and mechanical stabilities and to decrease the rate of drug release by employing natural or synthetic reagents such as glutaraldehyde [7].

ENR is a broad spectrum antimicrobial agent that has been developed as a drug for animal use. It has the maximal lipid solubility among fluoroquinolones and this property promotes its penetration into biological tissues [8]. ENR is currently FDA-approved for treatment of pets and farm animals in the United States [9]. The focus of the present study was to prepare and evaluate the physicochemical and antibacterial properties of a chitosan/β-GP system as implantable films for controlled delivery of ENR.

Results

In vitro drug release

The cumulative drug release (%) from the film formulations as a function of time were shown in Figures 1 and 2. The cumulative drug release (%) of F1, F2, F3 and F4 formulations were 72.6, 70.1, 90.5 and 82.4, respectively. Formulations F1 and F2 released the drug for 5 h but drug release from F3 and F4 extended over 96 and 168 h, respectively. Formulations F1 and F2 started the release of ENR within the first hour of the experiment. The release of formulation F1 in 0.5, 1 and 2 h were significantly lower than those of F2 (p < 0.05). Formulation F3 and F4 started the release of ENR in 2 and 3 h, respectively. The burst effect had been controlled in formulation F3 and F4 by changing the preparation method. The release of formulation F4 in 24, 48, 72 and 96 h was significantly lower than those of F3 at these time points (p < 0.05).

In vitro drug release kinetic model

The model fitting for the release profile of formulations was shown in Table 1. Based on the higher regression values (r²), the best fit model was Korsmeyer-Peppas for formulation F1 and F2, whereas Higuchi model for F3 and F4 formulations. The values of “n” were calculated from the drug release data (< 70%). The obtained values of formulation F1 and F2 were between 0 and 0.5, indicating that the release pattern of ENR was correlated to Fickian diffusion. These values for formulations F3 and F4 were > 1, indicating that the drug release was by super case II transport.
Table 1.
In vitro drug release kinetics parameters of different enrofloxacin films

<table>
<thead>
<tr>
<th>Film code</th>
<th>Zero order $r^2$</th>
<th>First order $r^2$</th>
<th>Higuchi $r^2$</th>
<th>Hixson-Crowell $r^2$</th>
<th>Korsmeyer-Peppas $n$</th>
<th>Korsmeyer-Peppas $r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.933</td>
<td>0.970</td>
<td>0.9822</td>
<td>0.960</td>
<td>0.45</td>
<td>0.9929</td>
</tr>
<tr>
<td>F2</td>
<td>0.782</td>
<td>0.819</td>
<td>0.8572</td>
<td>0.807</td>
<td>0.067</td>
<td>0.9073</td>
</tr>
<tr>
<td>F3</td>
<td>0.887</td>
<td>0.959</td>
<td><strong>0.9726</strong></td>
<td>0.939</td>
<td>3.43</td>
<td>0.7970</td>
</tr>
<tr>
<td>F4</td>
<td>0.960</td>
<td>0.704</td>
<td><strong>0.9920</strong></td>
<td>0.711</td>
<td>2.70</td>
<td>0.8755</td>
</tr>
</tbody>
</table>

Figure 3.
The FTIR spectra of A: ENR, B: Chitosan, C: β-GP, Chitosan/β-GP and formulation F1 are shown in figure 3.

Fourier transform infrared spectroscopy (FTIR) findings

The FTIR spectra of ENR, chitosan, β-GP, chitosan/β-GP and formulation F1 are shown in figure 3. The FTIR studies showed no chemical interaction between ENR and excipients used in the study. The ENR has two characteristic absorption peaks, 1736 cm$^{-1}$ and 1628 cm$^{-1}$; the first is the C=O vibration absorption peak from carboxylic acid oxygen, and the second is assigned to keto C=O peak.
from the ring of ENR. For the ENR film, the band 1628 cm\(^{-1}\) was shifted to 1635 cm\(^{-1}\), respectively.

In the wave number range 800-1200 cm\(^{-1}\), the FTIR spectrum of chitosan shows three bands at 1155, 1030 and 894 cm\(^{-1}\). The wide band at 1030-1155 cm\(^{-1}\) represents the bridge \(-\text{O-}\) stretch of the glucosamine residues in chitosan. The spectrum of chitosan shows a band at 1595 cm\(^{-1}\) that is assigned to the NH\(_2\) group of chitosan. These bands indicate that chitosan is a partially deacetylated product of chitin. The chitosan molecule shows four peaks at 1423, 1380, 1315 and 1255 cm\(^{-1}\). The bands at 1423 and 1315 cm\(^{-1}\) are associated with oscillations characteristic for OH and C-H bending of CH\(_2\) groups. The band at 1380 cm\(^{-1}\) represents the C-O stretching of the primary alcoholic group \(-\text{CH}_2\text{-OH}\). A weaker amino characteristic peak at 1255 cm\(^{-1}\) is associated with O-H bending vibration. Chitosan exhibited a broad peak at 3434 cm\(^{-1}\), which was assigned to the stretching vibration of N-H and O-H bond. Peaks at 2924 cm\(^{-1}\) were due to the C-H stretch vibrations. A peak at 1653 cm\(^{-1}\) was due to the C=O stretch of amide bond.

Two bands characteristic of GP appear at 971 cm\(^{-1}\) and 1076 cm\(^{-1}\), with a minor shoulder at 910 cm\(^{-1}\). The band at 1076 cm\(^{-1}\) is characteristic for GP and indicates the \(-\text{PO}_4^{2-}\) group. The band at 971 cm\(^{-1}\) is characteristic for the aliphatic P-O-C stretching and the band at 910 cm\(^{-1}\) may indicate the presence of the \(-\text{HPO}_4^{2-}\) group.

### Morphological findings

The appearance of films was creamy, smooth, non-sticky, homogenous and flexible. The SEM photographs of the samples are shown in Figure 4 (A-D). It showed that the surface of single layer film was rough, obviously dense, porous, homogenous and integrated. The triple-layer film also had a continuous and porous structure, but its texture was softer than single layer films, while the swelled film (F4) in phosphate buffer (pH=7.4) had a more porous structure than non-swelled films as shown in Figure 4 (D).

### Antimicrobial activities

The zones of inhibition of microbial growth provided by positive control and ENR films were presented in Figure 5 and Table 2. It showed that the inhibition zones of positive control and single layer films were significantly greater than those of triple-layer films \((p<0.05)\). However, no statistical differences in inhibitory zones were observed between the positive control and single layer films against \(S.\ aurus, P.\ aeruginosa\) and \(E.\ coli\). In addition, no inhibition zone was observed for the films without ENR (the blank formulations).

### Discussion

Infection control in animals is a primary clinical objective in veterinary field and is usually achieved by drug therapy, once or twice a day for at least 3-5 days. The objective of the present study was to prepare and

<table>
<thead>
<tr>
<th>Table 2. The zones of inhibition of microbial growth around the enrofloxacin films and the cylinder containing enrofloxacin suspension (positive control).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
</tr>
<tr>
<td>E. coli</td>
</tr>
<tr>
<td>P. aeruginosa</td>
</tr>
</tbody>
</table>

Note: Data represent mean ± SD (n=3). Different letters denote significant differences \((p<0.05)\).
evaluate ENR films as sustained release formulations. The polymeric matrix used in this study consisted of chitosan polymer and β-Glycerophosphate (β-GP). Glycerol was included in the films in order to achieve the desired plasticizing action.

Our results showed that formulation F2 had the highest burst release (65%) within 1 hour. Formulation F1 also exhibited a high burst effect (38%) within 1 hour, but there were no burst effects in formulations F3 and F4 within the first hours. In triple-layer films, following an initial burst release within 24 hours, drug was released gradually from chitosan films. The formulations F3 and F4 could sustain ENR delivery for 96 h and 168 h, respectively, which is a reasonable time span for antimicrobial therapy.

The rate of drug release was higher in F2 formulation in comparison to that of F1 formulation containing the same amount of chitosan but with different amounts of β-GP (750 mg in F2 and 500 mg in F1). This can be explained by the fact that the pore size increases with an increase in β-GP content [11] and the porous structure of the films are made by interactions between chitosan and β-GP. Moreover, the ENR molecules (with molecular weight of 359.4 Da) are small enough to move out through the pores of polymeric network. This might be the cause of faster drug release from F2 films as compared to F1 films [9, 12].

The strength and water preservation efficiency of the hydrogel depends on the amount of crosslinking agent used because the chitosan molecules can be transformed into polymeric network structure by the addition of crosslinking agent and consequently, water molecules can be retained in the network [13]. This issue correlates well with the results of the present study where the in vitro releases of drug were prolonged from the formulations F3 and F4 that contained glutaraldehyde in lower and top layer. These formulations could prolong the release of ENR for 4 and 7 days, respectively. The absence of peak in the 1720–1740 cm⁻¹ region indicated the absence of an aldehyde group in the cross-linked product and, hence, any residual unreacted GA. The cross-linked chitosan films showed peaks at 1572 cm⁻¹, indicating the formation of imine bonds due to cross-linking reaction of free amino groups of chitosan with the aldehyde groups of glutaraldehyde (GA). The spectra of chitosan cross-linked with GA showed typical aliphatic groups of GA at 1380 and 1315 cm⁻¹ were shifted to 1385 and 1319 cm⁻¹, respectively, but the bands of GP at 1076 and 971 cm⁻¹ were shifted to 1069 and 976 cm⁻¹

With regard to release kinetics, it has been proposed that drug release from matrices usually implies water penetration into the matrix, hydration, swelling, diffusion of the dissolved drug, and/or the erosion of the gelatinous layer. The release mechanism of a drug would depend on the selected dosage form, pH, and the nature of the drug and polymer [2]. In the present study, formulations F3 and F4 fitted best with Higuchi release model, in which the release rate decreased with time. The Higuchi model indicates a diffusion-controlled mechanism of release. The erosion of the polymer in formulation F3 and F4 could gradually reduce the diffusion path length, which in turn attenuates the reduction of the release rate in Higuchi model [14].

The cross-linked chitosan films showed peaks at 1572 cm⁻¹, indicating the formation of imine bonds due to cross-linking reaction of free amino groups of chitosan with the aldehyde groups of glutaraldehyde (GA). The spectra of chitosan cross-linked with GA showed typical aliphatic groups of GA at 1631 cm⁻¹ due to N=C bond and 1558 cm⁻¹ attributed to C=C bond. For the film formulation, the bands at 1558 and 1631 cm⁻¹ were shifted to 1562 and 1635 cm⁻¹, respectively. The absence of peak in the 1720–1740 cm⁻¹ region indicated the absence of an aldehyde group in the cross-linked product and, hence, any residual unreacted GA.

The various components of the formulations, especially the glycerol, resulted in loss of infrared features owing to severe background absorbance, and complicated the interpretation of spectra. Three basic infrared features of chitosan powder, namely the amide C=O stretch, amide N-H bend, and CH₃ scissoring modes, were compared with that of film. The C=O stretching frequency of amide was found to be lower in the film. This shift is attributed to hydrogen bonding with hydroxyl groups of glycerol, whereas shifts in N-H bending and CH₃ scissoring frequencies indicate altered interaction/mobility of polymer chains [15].

The morphological tests of the film formulations
showed similar properties as shown by other researchers such as Jiang et al. (2011) who fabricated bioabsorbable chitosan/β-GP composite membranes. They also reported rough surface and porous structure of the membranes caused by interaction between chitosan and β-GP [12].

Regarding microbiological studies, the findings indicated that the inhibition zones were significantly (p < 0.05) smaller for the triple-layer films as compared to the single layer films and the free drug (positive control). It seems that it is mainly due to slow drug release from triple-layer films in comparison to single-layer formulations during 24 hours. Aviv et al. (2007) also prepared polymer films consisting of poly (L-lactic acid) or poly (D, L-lactide-co-glycolide) and gentamicin for prevention of bacterial infections as-sociated with orthopedic implants. All films exhibited marked gentamicin release, which was responsible for the dramatic decrease in bacterial survival (10⁸/mL CFU after 24 h). Practically, no bacteria survived after 1–3 days, so the film preparation process did not affect the potency of gentamicin as an antibacterial agent [16]. The findings of the present study also showed that ENR maintained its antibacterial properties in the films and demonstrated the antimicrobial activity against all the bacterial strains tested.

In conclusion, the present study showed that the triple-layer chitosan/GP films were more effective than single layer formulations to deliver ENR for a long period and this approach represents an attractive technology platform for the delivery of other clinically important hydrophobic drugs. Both F3 and F4 formulations can be considered for further research and use in antimicrobial therapy to release ENR for 4 and 7 days, respectively. The major advantages of these systems include targeted local delivery of drugs at a constant rate such as postsurgical use to prevent infection.

### Materials and methods

**Materials**

Medium molecular weight chitosan with degree of deacetylation (DDA) of 75-85% and β-glycerophosphate disodium salt pentahydrate were purchased from Sigma-Aldrich (St. Louis, MO). ENR (99.57%, assay by HPLC) was obtained from TEMAD Pharmaceutical Co. (Tehran, Iran). Acetic acid, glutaraldehyde and glycerol were purchased from Merck (Darmstadt, Germany). Other chemicals were reagent grade.

**Preparation of the films**

The solvent evaporation technique was used for preparation of the films. The compositions of the film formulations of ENR have been presented in Table 3. To make single layer films (F1 and F2 formulations), a chitosan solution was prepared by stirring 200 mg powdered chitosan in 6 ml aqueous acetic acid (1%, v/v) and maintaining at 4°C overnight. The insoluble particles were removed by filtration. The chitosan solution was mixed with 100 mg ENR by stirring at room temperature for 2 h and 100 µl glycerol was added. Then, 500/750 mg β-GP (for preparation of F1/F2 formulation, respectively) along with 30 µl glutaraldehyde was dissolved in deionized water. The prepared chitosan and β-GP solutions were placed in an ice-water bath for 15 min and then the β-GP solution was added to chitosan solution drop-wise. Chitosan films were prepared by pouring the final 8 ml solution onto the Teflon molds.

The contents in the molds were left to dry at ambient temperature for 72 h to form the circular films. The dried transparent film was carefully peeled off from the plates, washed with distilled water, and then air dried (Figure 6).

The triple-layer films were prepared by pouring successive layers onto a mold in two ways. In the first way (F3 formulation), chitosan solution was prepared by stirring 150 mg powdered chitosan in 4 ml aqueous acetic acid (1%, v/v). This solution was maintained at 4°C overnight. Then, 500 mg β-GP along with 50 µl glutaraldehyde was dissolved in deionized water. The chitosan and β-GP solutions were placed in an ice-water bath for 15 min. The β-GP solution was added to chitosan solution drop-wise. Then, 100 µl glycerol was added to solution. The final 5 ml solution was cast as the first layer. The third layer was prepared similar to the second layer and the solvent was evaporated. The triple-layer films were dried at ambient temperature for 72 h to form circular films. The dried transparent films were carefully peeled off from the plates, washed with distilled water, and then air-dried (Figure 6).

### Table 3.
The compositions of the film formulations of Enrofloxacin.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan (mg)</td>
<td>200</td>
<td>200</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>β-GP (mg)</td>
<td>500</td>
<td>750</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Glutaraldehyde (µl)</td>
<td>30</td>
<td>30</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Glycerol (µl)</td>
<td>100</td>
<td>100</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>ENR (mg)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Note: Composition of triple-layer films (F3 and F4) are the same, but their preparation methods are different with regard to the method of the addition of second layer (see text).

**Materials and methods**

**Preparation of the films**

The solvent evaporation technique was used for preparation of the films. The compositions of the film formulations of ENR have been presented in Table 3. To make single layer films (F1 and F2 formulations), a chitosan solution was prepared by stirring 200 mg powdered chitosan in 6 ml aqueous acetic acid (1%, v/v) and maintaining at 4°C overnight. The insoluble particles were removed by filtration. The chitosan solution was mixed with 100 mg ENR by stirring at room temperature for 2 h and 100 µl glycerol was added. Then, 500/750 mg β-GP (for preparation of F1/F2 formulation, respectively) along with 30 µl glutaraldehyde was dissolved in deionized water. The prepared chitosan and β-GP solutions were placed in an ice-water bath for 15 min and then the β-GP solution was added to chitosan solution drop-wise. Chitosan films were prepared by pouring the final 8 ml solution onto the Teflon molds.

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to the first layer. The second layer was contained 100 mg chitosan dissolved in 4 ml aqueous acetic acid. The chitosan solution was mixed with 100 mg ENR by stirring at room temperature for 2 h. The only difference between two ways was how to pour the second layer in the mold. In the first way (F3), the second layer was poured on the whole surface of the first layer whereas in the second way (F4), the second layer was poured on the centre of the first layer. The first and the third layer left to dry for 48 h but the second layer left to dry for 24 h (Figure 7).

In vitro drug release studies

In vitro drug release tests were performed under sink conditions using a piece of film immersed at 37°C in 500ml of phosphate buffer, pH = 7.4, containing 0.5% Tween 80. The dissolution system was shaken at 100 rpm. Samples from dissolution medium were removed periodically and the medium was replenished. The absorbance of the samples was measured at 273 nm by using a UV-Vis spectrophotometer. All measurements were performed in triplicate.

In vitro drug release kinetic study

The drug release kinetic is directed by one or more mechanisms that depends on the composition of the matrix, geometry, preparation method and dissolution media. This can be explained by mathematical models in accordance with the desired or required predictive ability and accuracy of the model [17]. To assess the drug release kinetic, data were analysed per the zero order, first order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas models. The coefficient of correlation (r²) values were calculated for a linear curve obtained using regression analysis of the plots from these models [18].

Morphological Studies

For sample preparation, all formulations were initially placed in a freezer at -20°C for a short-term and then freeze-dried overnight. To study the morphology of formulations, dried films were cut with a sharp blade to expose internal microstructure and coated with platinum-gold for SEM imaging at 30 kV using scanning electron microscope (FESEM, Hitachi. S4160, Japan).

FTIR spectra

FTIR spectroscopy was used to assess the polymer chemical groups and investigate the formation of cross-linked networks through the reaction of chitosan with glutaraldehyde. FTIR spectra of ENR, chitosan, β-GP, dried chitosan/β-GP film, glutaraldehyde and formulation F1 were recorded in KBr pellets. To prepare 0.50-mm-thick KBr pellets, 3–5 mg of powder films (extra fine) mixed with 200 mg dried KBr. The FTIR spectra between 400-4000 cm⁻¹ were recorded using a FTIR spectrophotometer (Nicolet, Model Impact 410; Madison, WI) at room temperature.

Microbiological studies

To determine the antibacterial activity of ENR films, the “diffusion test” was carried out by using Escherichia coli, ATCC35218, and Pseudomonas aeruginosa ATCC10145 as gram-negative pathogenic strains and Staphylococcus aureus ATCC29213 as gram-positive pathogenic strain. The antimicrobial efficacies of all films were evaluated by placing films on the solid agar medium. Pieces of 0.5×0.5 cm of each ENR film (containing 20µg of ENR) were used as well as the blank preparation (formulated exactly the same without adding ENR). The blank formulation was used as a negative control to investigate the antimicrobial properties of chitosan. ENR suspension was also used as a positive control. Wells with 8mm diameters were prepared for delivery of aliquots of 20µl of positive control (containing 1µg/µl of ENR) into medium. After incubation for 24h at 37°C, the zones of inhibition around the wells were measured using a calliper [19]. All experiments carried out in triplicate.

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The authors wish to thank TEMAD Pharmaceutical Co. (Iran) for gift sample of enrofloxacin as well as Prof. Muhammad Reza Rouini for providing facilities for completion of this work. The authors also wish to express their gratitude to Mr. Iraj Ashrafi Tamai for his help in the microbiological tests and Dr. Pegah Khosravian for her assistance in this project.

Author contributions

Conceived and designed the experiments: SKF, AR, HAJ. Performed the experiments: SKF, KK. Analyzed the data: YHA, SKF. Provide research space and equipment: HAJ, TZS. Contributed reagents/materials/analysis tools: AR, YHA, TZS. Wrote the paper: AR, SKF.

Conflict of interest

The authors declare that they have no competing interests.

References

Preparation of chitosan-based films for enrofloxacin


Effect of *Zataria multiflora* essential oil on rooster semen during storage at 4°C

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Animal Science Department, College of Agricultural Science, University of Guilan, Rasht, Iran

**Keywords**
antioxidant, essential oil, rooster sperm

**Abstract**

Experiment was conducted to determine effect of *Zataria multiflora* boiss essential oil on stored spermatozoa. Semen collection was performed by using 15 mature roosters twice a week at four times. In each session, ejaculates were pooled and split into seven parts. The amounts of 0 (EO0), 50 (EO50), 100 (EO100), 200 (EO200), 400 (EO400), 600 (EO600) and 1000 (EO1000) ng/ml *Zataria multiflora* boiss essential oil were added to each part. Samples were chilled to 4°C and maintained for 72 h. Sperm assessment was performed at 0, 24, 48 and 72 h. Lipid peroxidation was evaluated after 48 h. Results showed that there was no interaction between *Zataria multiflora* essential oil and incubation time on membrane integrity, sperm motility and viability (*p > 0.05*). The highest sperm progressive motility (80.43%), viability (86.31%) and functional membrane integrity (85.81%) was observed in EO200 (*p < 0.05*). The lowest sperm motility (61.31%) and viability (73.31%) was observed in EO1000 (*p < 0.05*). The concentrations of malondialdehyde was lowest in EO200 (0.17 nM/ml, *p < 0.05*). Therefore, addition of 200 ng/ml *Zataria multiflora* boiss essential oil to semen improved longevity of rooster spermatozoa at 4°C.

**Abbreviations**

RSA: radical scavenging activity
EO: essential oil
MDA: malondialdehyde
HOST: hypo-osmotic swelling test
Introduction

Nowadays, livestock breeders use artificial insemination to reduce costs, control sexual diseases and accelerate genetic improvement of the herd [1, 2-3]. Artificial insemination is well used in dairy cattle, pigs, sheep and commercial turkey breedings, but the use of this method is limited in poultry breeding due to cost and semen storage problems [4]. Artificial insemination may become cost-effective in broiler breeder management, since it would be possible to increase the insemination interval to 10-14 days (instead of 7 days) with a lesser concentration of sperm per insemination [5]. It is well known that fertility following the use of artificial insemination will be satisfactory if the quality of the stored sperm is kept well. However, there is greater willingness to develop liquid semen storage methods for economic and practical reasons in the poultry industry [6].

Diluting and cooling semen is necessary to store spermatozoa in vitro. Semen dilution decreases the concentration of seminal plasma antioxidants. Plasma membrane of birds’ spermatozoa is rich in unsaturated fatty acids and phospholipids [7]. High concentration of polyunsaturated fatty acids in the sperm membrane causes extreme sensitivity to lipid peroxidation, being positively correlated with male infertility [8]. Supplementation of bird semen extenders with antioxidant may improve quality of the stored sperm.

Compounds in plant essential oils display antioxidant activity [9]. Moreover, these products seem to have lower side effects and can be regarded safer in cell biology analyses [10]. Zataria multiflora, known as Avishan Shirazi, is an aromatic plant whose anti-bacterial, antiviral, antifungal, acaricidal, and antioxidant activities have been demonstrated [11]. In addition, the essential oil of Zataria multiflora includes phenolic compounds, playing a role as scavengers of free radicals [12]. The objective of this experiment was to determine the effect of different levels of the essential oil of Zataria multiflora as a supplementation agent for standard extender (Sexton extender) on the longevity of refrigerated rooster spermatozoa.

Results

There was no interaction between Zataria multiflora essential oil and incubation time on membrane integrity, sperm motility and viability (p > 0.05). Membrane integrity, sperm motility and viability were higher in the group with 200 ng/ml essential oil than the control group (Table 1; p < 0.05). The lowest membrane integrity, sperm motility and viability were observed in the group with 1000 ng/ml essential oil (p < 0.05). There was no difference between the effects of 0, 50 and 100 ng/ml essential oil on membrane integrity, sperm motility and sperm viability (p > 0.05). Membrane integrity, sperm viability and motility were lower in the presence of 600 ng/ml essential oil than in the control group (p < 0.05). There was no difference between 0 and 400 ng/ml essential oil on membrane integrity (p > 0.05). The lowest MDA concentration was observed in the group with 200 ng/ml essential oil (Table 2; p < 0.05). Concentration of MDA was lower in groups with 50 and 100 ng/ml essential oil than the control group (p > 0.05). Concentration of MDA was higher in the groups with 400, 600 and 1000 ng/ml essential oil than the control group (p> 0.05).

Discussion

The spermatozoa metabolism does not completely stop at sub-ambient temperature, but its rate declines. Free radicals as toxic products of metabolism, may accumulate and damage sperm
structure and function during cold liquid storage. It would be expected that quality of stored spermatozoa improves through scavenging free radicals during semen storage [13].

We observed that supplementation of semen extender with *Z. multiflora* essential oil was effective on concentration of MDA in a dose-dependent manner. *Z. multiflora* essential oil up to 200 ng/ml inhibited sperm lipid peroxidation, while the amount of ≥ 400 ng/ml of essential oil increased the MDA concentration. Incubation of mammalian cells with compounds obtained from other plants such as epigallocatechin-3-gallate [14], quercetin [15], and silymarin [16], was accompanied by similar results. *Z. multiflora* contains high levels of phenolic compounds [11]. These polyphenols play important role in absorption and neutralization of free radicals, quenching singlet oxygen [17-18]. Moreover, it was reported that the *Z. multiflora* essential oil had a potent radical scavenging activity [11]. However, it was reported that the free radicals absorbing capacity of antioxidants increased with concentration, only when their concentration was low, thus, acting as a strong oxidation stimulator at high concentrations [19]. It seems that *Z. multiflora* essential oil may act as prooxidant affecting inner cell membranes and organelles such as mitochondria at high concentrations [11].

During the time of storage, membrane integrity, sperm viability and motility were declined, which was in agreement with study performed by other investigators [20]. Decrease in quality of stored spermatozoa in liquid form related to the accumulation of the toxic products of metabolism [21].

Sperm assessment showed that membrane integrity, sperm viability and motility were higher in 200 ng/ml *Z. multiflora* than the control group. Furthermore, the sperm quality decreased in the presence of ≥600 ng/ml *Z. multiflora* essential oil during cooled liquid storage. In the present study, we found a dose dependence of the effects of *Z. multiflora* essential oil on stored rooster spermatozoa in a cool liquid form. It has been shown that the exposure to essential oils could induce mitochondrial damage involving mitochondrial membranes and DNA [22]. However, it was reported that the use of the low level of herbal antioxidants such as resveratrol and quercetin improved sperm quality rate [23]. Furthermore, cell viability was reduced at high concentration (1000ng/ml) of *Z. multiflora* essential oil [11]. The main components of the essential oil of *Z. multiflora* were reported to be thymol (16%) and carvacrol (52%) and p-cymene (10%) [11]. It is well known that carvacrol has anti-oxidative and anti-apoptotic properties [24]. Low concentrations of carvacrol protected DNA from oxidative damage mediated by hydroxyl radicals from hydrogen peroxide, while its high concentrations increased DNA damage [25]. Additionally, thymol can interact with several proteins, phospholipids, cell membranes affecting membrane permeability, membrane potential and potassium fluxes [26]. Moreover, it was reported that some herbal volatile oils containing high level of thymol had spermicidal effects [27-28]. Thymol does not have protective effects on spermatozoa, and acts as potent immobilizing and spermicidal agent [29], whereas carvacrol, as one of the major components of the essential oil *Z. multiflora*, can protect sperm during storage. It has been suggested that the effects of thymol on sperm are partly masked by the effects of other compounds present in the essential oil [29]. It has also been mentioned that the low concentration

<table>
<thead>
<tr>
<th>Zataria multiflora essential oil (ng/mL)</th>
<th>Malondialdehyde (nM/10×10⁶ sperm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.81 ± 0.09</td>
</tr>
<tr>
<td>50</td>
<td>0.50 ± 0.09</td>
</tr>
<tr>
<td>100</td>
<td>0.49 ± 0.09</td>
</tr>
<tr>
<td>200</td>
<td>0.17 ± 0.09</td>
</tr>
<tr>
<td>400</td>
<td>0.17 ± 0.09</td>
</tr>
<tr>
<td>600</td>
<td>1.21 ± 0.09</td>
</tr>
<tr>
<td>1000</td>
<td>1.48 ± 0.09</td>
</tr>
</tbody>
</table>

Different letters (a-e) within a column shows significant differences (p < 0.05).
of flavonoid improved cell survival and reduced apoptotic function, while higher concentrations increased apoptosis [15]. Therefore, it is possible that protective effects of *Z. multiflora* essential oil at low concentration (up to 200 ng/ml) may be associated with proper concentration of monoterpenic phenol such as carvacrol. Moreover, it has been speculated that the toxic effect of thymol might decrease sperm quality at high levels of essential oil of *Z. multiflora*.

**Conclusion**

There was no interaction between *Zataria multiflora* essential oil and incubation time on the quality of rooster spermatozoa. *Z. multiflora* essential oil was effective on rooster spermatozoa in a dose-dependent manner. Supplementation of sperm extender with *Z. multiflora* essential oil up to 200 ng/ml improved quality of stored sperm, while the amount of ≥ 400 ng/ml of essential oil had detrimental effects.

**Material and methods**

**Zataria multiflora essential oil compounds**

<table>
<thead>
<tr>
<th>Table 3. Content of total phenols, flavonoids and antioxidant activity of <em>Zataria multiflora</em> essential oil</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>content/activity</strong></td>
<td><strong>RSA (%) = (Acont – Asamp)/Acont × 100</strong></td>
</tr>
<tr>
<td>Total polyphenols content (mg of Gallic acid/mL of essential oil)</td>
<td>2.229 ± 0.003</td>
</tr>
<tr>
<td>Flavonoid content (mg of catechin/mL of essential oil)</td>
<td>12.982 ± 0.04</td>
</tr>
<tr>
<td>Free radical scavenging activity (RSA %)</td>
<td>91.424 ± 0.034</td>
</tr>
<tr>
<td>Values are means of three replicates ± SD.</td>
<td></td>
</tr>
</tbody>
</table>

**analysis**

*Zataria multiflora* essential oil (Barij Essence Pharmaceutical Co, Iran) compounds analysis was performed using T80+ V/ Visspectrometer (PG Instrument, Ltd). Determination of each compound was performed in three replicates. Table 3 presents the results of essential oil compounds analysis. Total polyphenols content of *Zataria multiflora* essential oil was determined using the Folin-Ciocalteu method [30]. Total flavonoids content of *Zataria multiflora* essential oil was determined by the following procedure of Park et al. [31].

The free radical scavenging activity (RSA) of essential oil was determined by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay based on the method of Du et al. [11] with minor modifications. Briefly, 75 µL of essential oil was added to 925 µL of a 0.1 mM solution of DPPH in methanol. After the reaction was allowed to occur in the dark for 30 min, the absorbance at 517 nm was recorded to determine concentration of remaining DPPH. Inhibition of DPPH in percent (RSA %) of essential oil sample was calculated by the following formula:

\[
RSA (\%) = \left(\frac{A_{cont} - A_{samp}}{A_{cont}}\right) \times 100
\]

where Acont is the absorbance of control reaction, and Asamp is the absorbance of the tested sample.

**Semen collection and preparation**

Fifteen healthy fertile native Guilan roosters at the age of 32 weeks were used. The birds were kept in individual cages (1×1×1.5 m) for 10 weeks. They were also kept under uniform husbandry conditions with 14 h light/day, 80 (at beginning) to 90 (up to end) g/day food (protein: 12.7 %; energy metabolism: 2760 Kcal/Kg; Ca: 1.2%; P: 0.4 %) and water ad libitum. The animals kept and cared for under experimental procedures and protocols approved by the Veterinary Organization of Iran and were housed at the University of Guilan, Faculty of Agricultural Sciences, Education Research and Practice Farm, South of Rasht (it is located at 37°12' north latitude and 49°39' east longitude).

*Zataria multiflora* essential oil was dissolved in absolute ethanol (1 mg/ml) and then diluted up to 2 pg/ml by Sexton extender (0.64 g potassium citrate tribasic monohydrate, 8.07 g sodium-L-glutamate, 0.34 g magnesium chloride anhydrous, 5 g d(-)-Fructose, 12.7 g potassium phosphate dibasic trishydroxide, 0.65 g potassium phosphate monobasic, 3.95 g TES, 4.3 g sodium acetate trishydrate, 1 l distilled water, pH 7.3–7.4).

Semen samples were collected by abdominal massage with three-day intervals between sessions over six consecutive weeks. After ejaculation, the semen was diluted 0.5: 1 (v/v) with Sexton extender. The samples were immersed in 39°C water and transferred to the laboratory by Styrofoam box within 10 min after collection. Upon reaching the laboratory, the evaluation of the samples was performed immediately. All diluted ejaculates were tested to possess acceptable progressive motility (>70%) and concentration (>3 × 10^6 sperm/ml).

In each session, the ejaculates (at least 10 collected ejaculates) were pooled and diluted to 4000 × 10^6 sperm/ml by Sexton extender. Diluted semen was split into seven parts and 0, 100, 200, 400, 600 and 1000 ng/ml *Zataria multiflora* essential oil were added. The final concentration of spermatozoa was 2000 × 10^6 cell/ml. The samples were cooled by Test Chamber (EG53AH, KATO, Saitama-ken, Honshu, Japan) to 4°C over 2 h (0.25°C/min) and incubated for 72 h. Sperm viability, motility and membrane integrity were evaluated at 0, 24, 48 and 72 h. After 48-hour incubation, lipid peroxidation level of sperm was measured by determining the malondialdehyde (MDA) production, using thiobarbituric acid [32]. Quantification of thiobarbituric acid reactive substances was determined by comparing the absorption with the standard curve of MDA equivalents generated by the acid catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane (Sigma Aldrich, USA). The values of MDA were expressed as nM/10 × 10^6 sperm/ml.

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**Sperm assessment**

The concentration of spermatozoa was determined by means of a Neubauer haemocytometer. Spermatozoa viability was assessed by the fix vital stain method [13]. The sample was mixed with an equal volume of glutaraldehyde fixative solution (glutaraldehyde at 2% in phosphate buffered saline). Then, it was mixed with an equal volume of 20 µg/ml bisbenzimide H33258. A smear was prepared after 10 min of incubation at room temperature. Two hundred spermatozoa per smear were evaluated in three to seven different microscopic fields for each sample using an Olympus IX70 phase-contrast microscope (high-pressure mercury illumination, UG1 excitation filter, U dichroic mirror, L420 barrier filter; Olympus, Tokyo, Japan). The procedure was performed by epifluorescence microscopy combined with bright-field illumination. Light intensity of the microscope was set at an optimum to
visualize both spermatozoa and fluorescence of H33258-labelled nuclei. Sperm showing partial or complete blue color were considered dead, and colorless sperm were considered to be alive (Figure 1).

The percentage of sperm motility was assessed by phase-contrast microscopy (400× magnification) at a warm stage at 37°C. The samples were diluted with extender up to 300 × 10⁶ sperm/mL, and a wet mount was made using a 5 μL drop of semen placed directly on a microscope slide and covered by a cover slip. Sperm motility was estimated at least 5 different microscopic fields for each semen sample. The subjective estimations were approximated to the nearest 10% by single technician. The mean of the successive estimations was recorded as the final motility score.

The hypo-osmotic swelling test (HOST) was used to evaluate functional integrity of the sperm membrane. HOST was performed by incubating 5 μL of semen with 500 μL of a 100 mM NaCl hypo-osmotic solution (7.35 g sodium citrate dihydrate and 13.51 g fructose in 1 L distilled water) at 37°C for 30 min. One drop of the mixture was placed on a pre-warmed slide, covered with a cover slip and examined under a phase-contrast microscope (400× magnification). The sperm with swollen tails were considered intact. To assess the percentages of intact sperm, a total of 200 sperm in at least five different microscopic fields were evaluated.

Statistical analysis

Analysis of variance was performed to study effects of the treatments on motility, viability and plasma membrane integrity of spermatozoa using MIXED procedure of SAS (2002) with repeated measures data. The samples taken from pooled semen were considered as subjects in these experiments. Statistical model included concentration of Zataria multiflora essential oil, time and their interaction effect. Results of MDA concentration were analyzed by using the GLM procedure. When the analysis revealed a significant difference, comparison of treatment means was performed by Tukey's test. For all statistical tests, the level of statistical significance was chosen at p < 0.05.

Author contributions

FG performed the experiments and MRAM designed the research project and wrote the draft of manuscript.

Conflict of interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

None of the authors have any conflict of interest to declare.

References


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21- During the time of storage, membrane integrity, sperm viability and motility were declined, which was in agreement with study performed by other investigators [20]. Decrease in quality of stored spermatozoa in liquid form related to the accumulation of the toxic products of metabolism [21]


Relationship between fructosamine, glucose, total protein, and albumin concentrations of serum in late pregnancy and early lactation of dairy Saanen goat

Roya Pourmohammad, Mehrdad Mohri, Hesam A. Seifi

Keywords
albumin, fructosamine, glucose, Saanen goat, total protein

Abstract
The changes in serum fructosamine concentration of dairy Saanen goats during late pregnancy and early lactation and its relationship to serum glucose, total protein, and albumin concentrations was investigated. Eleven Saanen goats were selected for the study. Blood samples were collected by the jugular vein in 30, 15 and 7 days before the expected time of parturition (D-30, D-15, and D-7, respectively) and also 12 hours and 3, 7, 13, and 42 days post-partum (H+12, D+3, D+7, D+13, and D+42, respectively). The serum concentrations of fructosamine, glucose, albumin, and total protein were measured. The maximum concentration of fructosamine was at 12 hours post-partum and decreased thereafter. Serum concentration of glucose significantly increased from D-15 up to 12 hours post-partum and then decreased and stayed at the same level. The serum concentrations of albumin and total protein significantly increased during post-partum period. There were significant correlations between fructosamine and glucose concentrations at post-partum period and overall time of the study. Linear regression analysis between each sampling amount of glucose and three consecutive fructosamine concentrations revealed significant positive correlation between glucose of each sampling time with the fructosamine of first sampling time thereafter. According to the correlation between glucose and fructosamine amounts during the study, especially post-partum period in Saanen goat, fructosamine measurement could be used as a useful indicator of energy economy and probably stress in Sannen goat; but controversy between reports in ruminants needs further studies for better understanding of fructosamine as energy biomarker in ruminants.

Abbreviations
Glu: glucose
Fruc: fructosamine
Alb: albumin
TP: total protein

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Introduction

Energy economy during late pregnancy and early lactation has considerable effect on the health and productivity of ruminants. In late pregnancy and early lactation, a decrease in blood glucose (Glu) will help hypoglycemia to develop which is a risk factor for metabolic disorders. Ketosis, fatty liver and pregnancy toxemia are the most important diseases near parturition in ruminants [1]. Also, pregnancy toxemia in dairy goats occur in the condition of energy deficiency, especially when it is accompanied by stress and multiple births [2]. The most important factor in the negative energy balance in goat and sheep is the increased need to energy, especially in the last month of pregnancy [3]. As a result, because of the important role of Glu in the metabolic process, the Glu measurement is appropriate in monitoring of herds metabolic status. Since the Glu concentration is dependent on other factors such as stress, daily activity, nutrition, and drugs, it is not a reliable marker for the evaluation of energy status in long periods [4].

Fructosamine (Fruc) is a glycated protein that is formed by the non-enzymatic and irreversible reaction of carbonyl group of sugar and free amino-group of protein and then it will be isomerized to a stable ketoamine [5]. The amount of glycation depends on the average blood Glu concentration as well as half-life of the blood proteins [6]. Synthesis of Fruc requires at least 2-3 weeks, so does not relate to the actual Glu concentrations. According to the average half-life of the protein in different species, the Fruc concentrations reflect the serum Glu concentration over the previous 1-3 weeks [7]. Serum Fruc concentration is proved to be an indicator of persistent hyperglycemia, e.g. diabetes mellitus, in humans, dogs and cats [8]. There are numerous articles concerning the use of Fruc in small animal practice and also some studies describing the usage of Fruc as part of the metabolic profile in dairy cows, ewes, and mares [6, 9, 10, 11, 12, 13]. The relationship between Fruc and Glu amounts in dog was previously reported [14, 15]. On the other hand, no correlations were reported in transition dairy cow and late pregnant sheep [12, 13]. Similar controversy was also existed concerning the relationship between Fruc, albumin (Alb), and total protein (TP) concentrations [12, 13, 16]. There is limited information about the use of Fruc in dairy goats as energy biomarker. In addition, negative energy balance is an important subject in dairy breeds of goat. Thus, it is important to investigate the physiological changes of Fruc and its relationships with other biochemical variables (TP, Alb and Glu concentrations) for determining the value of Fruc measurement as diagnostic and/or prognostic energy biomarkers of Saanen goats during late pregnancy and early lactation period.

Results

The mean ± SE of Fruc for all goats during the study was 314 ± 5.36 μmol/l. The changes of serum Fruc, Glu, total protein, and Alb concentrations during the time of study are shown in figures 1 and 2. There was no significant difference in the serum concentration of Fruc pre-partum, post-partum, and throughout the study. Significant differences in the amounts of Alb were seen pre-partum, post-partum, and throughout the trial. TP concentrations were significantly changed post-partum and throughout the study, but not at pre-partum period. The maximum concentration of Fruc was at 12 hours post-partum and decreased thereafter. Serum concentration of Glu significantly increased from D-15 up to 12 hours post-partum and then decreased and approximately stayed at the same amounts. The serum concentrations of Alb and TP significantly increased during post-partum period.

There were significant correlations between Fruc
and Glu concentrations at postpartum period and overall time of the study ($r = 0.37, p = 0.006$ and $r = 0.26, p = 0.02$, respectively) but no significant correlation was seen at pre-partum period. There were not any correlations between Fruc, total protein, and Alb amounts at pre-partum, postpartum and overall time of the study. Within the same sampling time, only the Glu and Fruc amounts of D+13 has significant correlation ($r = 0.81, p = 0.005$). From the retrospective viewpoint, Pearson analysis revealed significant correlations between the concentrations of Glu and Fruc at D-7 with H+12 ($r = -0.72, p = 0.019$), D+3 with D+7 ($r = 0.669, p = 0.024$), and D+3 with D+42 ($r = 0.724, p = 0.012$). Linear regression analysis between each sampling amount of Glu and three consecutive Fruc concentrations revealed significant positive correlation only between Glu with Fruc of first sampling time thereafter (Figure 3).

**Discussion**

Several reports recommend the usage of Fruc as part of the metabolic profile in dairy cows, ewes and mares [10, 12, 13, 17]. In the present study the cumulative mean ± SE of Fruc during the trial in 11 Saanen goats was $314 ± 5.36 \, \mu mol/l$. Cantley et al. [18] reported that the concentration of Fruc in 67 late pregnant sheep was $172 ± 2 \, \mu mol/l$ (mean ± SE). Sorondo and Cirio [13] reported that in late-pregnant Corriedale sheep during 11 weeks of study, the concentration of Fruc was $272 ± 83 \, \mu mol/l$ (mean ± SD). In another study on 10 crossbred dairy ewes, the concentrations of Fruc were between 142 to 156 \, \mu mol/l during 10 days before lambing to 130 days post-partum [17]. The results of our study were in agreement with the previous report for Corriedale sheep and dairy cows [12, 13], and higher than findings of other studies for
Fructosamine measurement in Saanen goat

sheep and dairy cows [17, 18, 19]. Probably, composition of diet, rate of protein turn-over during gestation and lactation, and difference in Glu metabolism or negative energy balance were responsible for this controversy.

In the present study Fruc and Glu concentrations showed similar time related changes at pre- and post-partum periods. This was in contrast with Sorondo and Cirio [13] and Filipović et al. [17] reports. Filipović et al. [17] suggested that the reduction in the amount of Fruc at 10 days after lambing is probably due to the concurrent reduction in the amount of Alb and TP. Based on their report, the amounts of TP and Alb progressively decreased during lactation period but Fruc did not follow similar pattern as Alb or TP. In our study, despite the reduction of Fruc from 12 hours to two weeks after the parturition, the serum concentrations of Alb and TP were significantly increased, so the reduction in the amount of Fruc cannot be related to the amounts of serum protein and Alb. According-like, we did not find any correlation between Fruc and Alb or TP concentrations in Saanen goats. Sorondo and Cirio [12, 13] reported similar findings in dairy cow and sheep, but Mostafavi et al. [19] reported significant correlation between Fruc and Alb amounts in 506 cows.

There are numerous studies that suggested a correlation between Fruc and Alb or TP amounts in dogs. Coppo and Mussart de Coppo [20] found that the concentration of Fruc in normoglycemic dogs with chronic hypoproteinemia is below the reference interval and so the density of serum Fruc can be affected by changes in blood protein concentration. Loste and Marca [16] reported high correlation in hypoproteinemic and hypoalbuminemic dogs between Fruc with TP and Alb concentrations. Jensen [21] in dog suggested that chronic hypoproteinemia or hyperproteinemia could affect the concentration of serum Fruc. It seems that further studies with more number of animals will be needed for better understanding of Fruc changes during late pregnancy and lactation in dairy goats and sheep.

In the present study, there were significant correlations between Fruc and Glu concentrations at post-partum period and overall time of the study. Linear regression analysis revealed significant positive correlation only between Glu with Fruc of first sampling time thereafter. Our result is consistent with biochemical pathway resulted to formation of Fruc and is in agree with previous reports for small animals [4, 9, 14, 15], dairy cows [19], and sheep [18]. On the other hand, Sorondo and Cirio [12, 13] reported no correlation between Glu with Fruc in retrospective approach in sheep and cow. The authors believed that in ruminants, fluctuations of Glu concentration are lower than human and domestic carnivores in which Glu amounts and probable changes are higher during Fruc formation. Our results are in contrast with those because correlation between Glu and Fruc was seen along with normal blood serum Glu concentration and without any relations to TP and Alb amounts as previously described. In addition, most correlations were observed during post-partum period. It seems metabolic challenges primarily negative energy balance due to milk production in this dairy breed of goat at post-partum period are responsible for Glu and Fruc relationship.

**Conclusion**

According to the correlation between Glu and Fruc amounts during the study, especially in the post-partum period in Saanen goat, Fruc measurement could be used as a useful indicator of energy economy and probably stress in Sannen goats. However, to resolve the controversy between reports in ruminants and for better understanding of Fruc as an energy biomarker in dairy cows, sheep, and goats, further studies will be needed.

**Materials and methods**

The study was performed on 11 dairy Saanen goats aged between 2 and 9 years of age. The does were synchronized for estrus by using two injections of PGF2α 11 days apart. Rams were introduced to does 3 days after the second dose. The rams remained with does for one week. Pregnancy was diagnosed by ultrasonography on days 60 and 80 after mating using a portable B-mode ultrasound scanner. The number of fetuses was recorded during the ultrasonographic diagnosis of pregnancy. The health of all goats was examined by clinical examination and CBC analysis before the beginning of the study. The animals were treated using ivermectin (0.2 mg/kg) and triclabendazol (10 mg/kg) for parasite control. All animals transferred to individual pens and were fed twice daily and fresh water was available ad libitum. The diets were formulated to meet all nutrient requirements for pregnant does at prepartum and lactating does during the postpartum period [22]. The ingredients of prepartum and postpartum diets are shown in Table 1. Daily diet of each goat was weighed prior to feeding and feed refusals were measured by digital scale weigher for accurate estimation of daily DMI of each animal. Blood samples were collected by the jugular vein in 30, 15 and 7 days before expected time of parturition (D-30, D-15, and D-7, respectively) and also 12 hours and 3, 7, 13, and 42 days post-partum (H+12, D+3, D+7, D+13, and D+42, respectively). All blood samplings were taken 3 h after first daily meal at the same time of the day for all animals. Blood samples were immediately transferred to the laboratory on ice. After clotting and centrifugation (1800g for 10 min) the serum was aspirated and stored at -20°C. The concentrations of Fruc (Nitroblue tetrazolium), Glu (Glu oxidase), Alb (Bromocresol green), and TP (Biuret) were measured with automated biochemical analyzer (BT 1500, Biotecnica instrument.
Acknowledgments

This work was supported by Ferdowsi University of Mashhad, grant number 3/20224.

Author contributions

Conceived and designed the experiment: MM, HAS. Performed the experiments: RP, MM. Analysed the data: MM, HAS. Research space and equipment: MM. Contributed reagents/materials/analysis tools: MM. Wrote the paper: RP, MM.

Conflict of interest

The authors declare that they have no conflict of interest.

References


9. Denis A.E., Richard W.N., Claudia E.R.: Comparison of serum Fruc and blood glycosylated hemoglobin concentration for Rome, Italy), using commercial kits (Pars Azmoon, Tehran, Iran for Glu, Alb, and TP, BioSystems, Barcelona, Spain for Fruc). Measurement accuracy was checked by using control serum (Randox control sera, Antrim, UK). All measured variables had a within-run coefficient of variation less than 10% (Total protein: 0.95%, Alb: 0.91, Glu: <3%).

Statistical analysis was done using SAS (The SAS system for Windows, version 9.1, Cary, NC). The skewness and kertosis of data were used for normality test. A repeated measure ANOVA was used to assess the effect of time of sampling throughout the study, pre-partum, and post-partum. The correlation between serum amounts of Glu, total protein, and Alb of each sampling time and Fruc of similar time, and three consecutive sampling time was determined with the help of linear regression and Pearson test [12]. All differences were considered to be statistically significant at $p < 0.05$.

Table 1.

<table>
<thead>
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<th>Ingredients</th>
<th>Pre-parturition</th>
<th>Post-parturition</th>
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<tbody>
<tr>
<td>Feed intake (kg DM /d)</td>
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<td>DMI (% BW)</td>
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<table>
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<tr>
<th>Ingredients (% DM)</th>
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<tr>
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<tr>
<td>Wheat bran</td>
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<table>
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<table>
<thead>
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<tr>
<td>Energy intake ME (Mcal/d)</td>
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</tr>
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<td>ADF (% DM)</td>
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<tr>
<td>NDF (% DM)</td>
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<td>Ca (% DM)</td>
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The hematological profile changes in Saanen goat kids from birth to 3 months of age

Safoora Abdolvahabi, Mahdieh Zaeemi, Mehrdad Mohri, Abbas Ali Naserian

Keywords: age, blood, goat kids, neonatal period, Saanen goat, small ruminants

Abstract

This study performed to determine hematological parameters in Saanen goats’ kids from birth until 3 months of age. The whole blood specimens were collected from 20 clinically healthy goat kids (15 females and 5 males). The blood specimens were obtained at 24-48h after birth and 10 ± 2, 28 ± 2, 56 ± 2 and 84 ± 2 days. Hematological variables were determined based on reference laboratory methods. Results showed significant age related changes for most factors (p < 0.05) except for MCHC and the number of monocytes, eosinophils, and band neutrophils. There were significant differences with first sampling time for all parameters (p < 0.01) except for the number of monocytes, eosinophils and band neutrophils. The results of the present study ould be used as reference values for proper interpretation of laboratory results.

Abbreviations

ARI: adult reference interval
EDTA: ethylene diamine tetra acetic acid
Hgb: hemoglobin
MCH: mean cell hemoglobin
MCHC: mean cell hemoglobin concentration
MCV: mean cell volume
N: L: neutrophil to lymphocyte
PCV: packed cell volume
RBC: red blood cells
WBC: total white blood cells
Introduction

Saanens are the largest of the goat dairy breeds. They are white or light cream in color. Their coats are usually short and fine; although a fringe over the spine and thighs is often present [1]. The name of this species originated in the Saanen valley in Switzerland. It is now the most popular dairy goat breed in many countries because of their heavy milk production, high reproductive yield, multi parturition, high growth and puberty rates [2-4]. The Saanen temperament is as a rule, calm and Saanen does are known for their ease of management in herds, and easily adaptation to different places [5-6].

Diseases of the newborn and neonatal mortality are major causes of economic loss in livestock production. Specific hematological reference ranges could help realistic evaluation of management practices, nutritional status and health conditions. Although there are reports concerning the values of serum hematological variables in other breeds of goats kids [7-11], but so far, the age related changes of hematological variables have not been studied in Saanen kids. Albeit these variables in Saanen goat kids, just has been com-

Figure 1.
Dot plots of hematological variables (○) and their medians (●) in kids from 1 to 84 days of age (Gray shading indicates adult reference intervals; Non-similar letters indicate significant difference (p < 0.01)).
pared with adults and were not investigated in growing animals [1].

Since age has profound effect on the amounts of many hematological factors [12,13], the aim of the present study was to investigate the physiological pattern of hematological variables by sequential measurement in growing goat kids in order to evaluate the need for defining reference values for different age groups. The obtained data should help in interpretation of laboratory results.

Results

Sampling time (age) had a significant \((p < 0.05)\) effect on most measured variables except eosinophil, band neutrophil and monocyte counts. The significant differences \((p < 0.01)\) between all times are presented in Figure 1.

PCV and Hgb showed a declining trend from 24-48 h to day 10 and thereafter increased up to day 84. MCV and MCH showed a decreasing trend throughout the study, while RBC, WBC and lymphocyte counts had an increasing trend. Neutrophil counts, N: L ratio and MCHC fluctuated in similar pattern. These variables decreased slightly from birth to day 10, thereafter increased up to day 56, and then showed a slight decreasing trend up to 3 months of age.

Discussion

Considering the significant impacts of environmental conditions on blood variables, investigation of hematological profile appears to be essential in growing goat kids during the first 3 months of life. In this study, the PCV and RBC were within adult reference range (ARI). Hgb concentration also was in ARI throughout this study except in day 10 that was in lower limit of ARI. Similar findings have been reported in lambs during the 70 days of age [14]. Hematological profile in neonatal calves is controversial. Knowles et al. 2000 showed that all these variables were within ARI in calves during the first three months of age [13], while in another study Hgb concentration was less than ARI until 2 months of age [12]. The environmental and breeds differences likely create this discrepancy.

Our results showed an increasing trend in RBC up to day 84. After declining of PCV and Hgb from 24-48 h to day 10, an increasing trend was also observed in these variables up to day 84. This pattern that is similar to hematological findings in dwarf and landrace kids [7] is likely related to elevated plasma volume due to colostral protein intake and rapid expansion of vascular space [15]. The failure of neonatal bone marrow in erythropoiesis also leads to this hemogram, because erythropoietin in neonatal period is not adequately produced by the underdeveloped neonatal kidneys [11].

MCV and MCH indices of kids were higher than that of adults from birth to day 10 and MCHC was lower than ARI during the first three months of age. Erythrocyte indices in newborn calves were below the ARI and decreasing the erythrocyte size has been observed until first 3 months of life [12]. In the blood specimens of the 70 days old lambs, the higher MCV and lower content of MCH and MCHC were observed in comparison with the 30 days old lambs [14].

In contrast to decreasing trend in MCV and MCH indices, MCHC showed little fluctuation throughout this study. The decreasing trends in MCV and MCH with advancing age have been reported by previous studies [7-10, 16]. The erythrocyte indices changes may be attributed to iron content or availability of diet and/or physiologic status during interpretation of erythrogram of neonates [12]. The amount of serum iron can differentiate these conditions from each other. Although serum concentration of iron in kids showed decreasing trend after day 28, it was within ARI during this study [17].

The presence of immature erythrocyte may be a cause of higher MCV in kids during first month of age. Neonatal erythrocyte size varies with the type of Hgb they contain. At birth, high percentage of the neonatal Hgb is Hgb F and there are higher numbers of reticulocytes in blood specimens which have larger size than mature RBCs [18]. Furthermore, the reduction of PCV, RBC, Hgb and MCH during the first days of life is probably due to replacing of reticulocytes and diffusely basophilic polychromatophilic erythrocytes by mature RBCs containing Hgb A [7].

After birth, WBC and lymph counts increased which is in agreement with other studies [8, 11, 19] and indicate maturation of immune system and exposure to pathogens that lead to cellular immune responses [19].

WBC count was higher than ARI after day 28. This is in contrast to previous finding that reported age related decrease of leukocyte count in Saanen goats [1]. This must be noticed that in the mentioned study, hemogram was compared between kids and adults not in growing kids. Furthermore, the environmental differences likely create this discrepancy. It was reported that WBC number of calves at birth tended to be above the upper limit of ARI but will drop to levels within ARI after that [13, 20]. The high levels in the WBC count would typically be attributed to physi-
Hematology of Saanen goat kids

Materials and methods

Twenty goat kids (15 females and 5 males) were blood sampled from birth to 84 days of age. A blood specimen was taken within 24 – 48 (± 2) hours, and at 10 ± 2, 28 ± 2, 56 ± 2, and 84 ± 2 days of age. The farm was monitored by the Veterinary School of Ferdows University of Mashhad and kid’s health status was evaluated before each sampling, based on rectal temperature, heart and respiratory rate, and other routine factors. Kids that showed abnormal signs in coughing, nasal discharge, ocular discharge, appetite status, and fecal consistency were excluded from the study.

Blood sampling was performed through jugular vein between 6.00 and 10.00 AM. Specimens were collected in K3-EDTA tubes (FL medical, Italy) and were placed immediately on ice for transferring them to the laboratory. Hematological variables such as RBC, Hgb, PCV, MCV, MCH, MCHC, total WBC, differential cell count (neutrophil, lymphocyte, eosinophil, monocyte and neutrophil to lymphocyte (N: L) ratio) were measured. All CBC related determinations were performed manually based on routine laboratory methods [25] except of total WBC count and Hgb concentration that was determined by a hematology analyzer (Nihon Kohden, Cell Tac a, MEK 6108, Tokyo, Japan).

Statistical analysis

Statistical analysis was conducted using SPSS for Windows (release 20, IBM, USA). Age effect was examined using non-parametric Friedman test. In addition, a non-parametric paired t test was used for the comparison of each sampling stage with first sampling time. Because of using multiple comparison test, the corrected p value was calculated and adjusted at 0.01. For each variable, age related changes were showed by a graph with upper and lower limits of adult reference intervals [18].

Acknowledgements

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Author contributions

Conceived and designed the experiments: MZ, MM. Performed the experiments: MZ, SA. Scientific counseling: AAN. Wrote the paper: MZ, SA.

Conflict of interest

None of the authors have any conflict of interest to declare.

References


Occurrence, hematologic and serum biochemical characteristics of neonatal isoerythrolysis in Arabian horses of Iran

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Keywords
neonatal isoerythrolysis, hemolytic anemia, Arabian horses, Khouzestan

Abstract
Neonatal isoerythrolysis is a major cause of anemia in newborn foals. However, there are no documented data regarding the occurrence of neonatal isoerythrolysis in Arabian horses of Iran, which are mostly raised in Khouzestan province. Hence, this study was carried out to investigate the occurrence of neonatal isoerythrolysis in Arabian horses of Khouzestan and assess the hematologic and serum biochemical profile of affected foals. A total of 20 neonatal foals, under one week of age, and their dams were involved in this study. Clinical examinations revealed no abnormality except in one foal with icteric mucous membranes, lethargy, tachycardia, tachypnea, hypothermia and hemoglobinuria which led to death. The diagnosis of neonatal isoerythrolysis was made based on erythrocyte agglutination in cross-match test between the mare serum and foal erythrocytes with the titer of 1:128, while other studied cases were assumed negative according to the test results. In the laboratory assessment, the foal with hemolytic anemia showed a major decline in hematocrit, hemoglobin concentration and erythrocyte count along with considerable leukocytosis and neutrophilia. Serum total and direct bilirubin concentrations in the NI case was about ten times higher than in the rest of the foals. This study revealed that neonatal isoerythrolysis can occur in Arabian foals of Khouzestan and is associated with severe anemia and icterus which may lead to death. These findings can be beneficial in the establishment of preventive measures in Arabian horse breeding industry in the region, as well as improving therapeutic methods.

Abbreviations
NI: neonatal isoerythrolysis
EDTA: ethylenediaminetetraacetic acid
PBS: phosphate buffered saline
RBC: erythrocyte count
HCT: hematocrit
HGB: hemoglobin concentration
MCV: mean corpuscular volume
MCHC: mean corpuscular hemoglobin concentration
WBC: leukocyte count
AST: aspartate aminotransferase
ALP: alkaline phosphatase
GGT: gamma-glutamyl transferase
Introduction

Neonatal isoerythrolysis (NI), as an acute hemolytic disease, is considered as one of the most important causes of anemia in neonatal foals. The disease originates from maternal alloantibodies directed against foal erythrocyte surface antigens [1], which are produced following exposure of mare to incompatible blood groups (blood leakage from placenta during pregnancy/parturition or incompatible blood transfusion). Over 30 equine erythrocyte antigens have been identified that produce 8 blood groups, among which, Aa and Qa are responsible for the majority of NI cases [2,3,4]. However, other erythrocyte antigens may also be involved [5]. Maternal antibodies of IgG type cannot transfer intact to the foal blood circulation through colostrum ingestion within the first few hours of birth. If the foal inherits erythrocyte antigens from the sire that the mare does not possess, NI would be possible in which maternal antibodies will bind offspring erythrocytes leading to intravascular and extravascular hemolysis [2].

Neonatal foals are normal at birth, but the symptoms will develop within 12 to 48 hours which may include lethargy, tachypnea, tachycardia, pale mucous membranes, icterus, hemoglobinuria, shock and even death, depending on the intensity of erythrocyte destruction and anemia. In addition, neurological signs, metabolic acidosis, toxic hepatopathy and dysfunction in various organs might be observed as a consequence of hypoxemia and tissue hypoxia. Furthermore, severe rise in non-conjugated bilirubin can result in encephalopathy or kernicterus in the involved newborn foals [3].

The disease should be considered in multiparous mares or mares with the history of previous foals with NI, although it cannot be completely ruled out in the first foaling. NI can be prevented in two stages: 1) Identification of the foals at risk through blood typing of both parent horses or cross match between them before parturition, and 2) Prevention of foals from exposure to the mare antibodies via prohibition of the neonate from nursing by its mother during the first 30 to 48 hours [1]. Identification of erythrocyte-bound antibodies in foal blood is essential for NI diagnosis which is performed by hemolytic or agglutination tests [6,7].

Epidemiologic studies revealed that around 14% of foals illustrate incompatibilities in red blood cell antigens with the dam [8]. However, not all incompatible pregnancies result in alloimmunization and NI [8]. The disease prevalence differs between various horse breeds [4] and has been reported to be approximately 2% in Standardbreds and 1% in Thoroughbreds [9], while its occurrence is higher in mule foals due to the presence of specific donkey erythrocyte antigens [10].

There are no documented data regarding the occurrence of NI in Arabian horses of Iran which are mostly raised in Khouzestan province, South-West Iran. Taking into account the importance of horse breeding industry in Khouzestan, besides the critical complications of NI in neonatal foals which was occasionally suspected in previous clinical observations in the region, it seemed necessary to perform a study in this field. Hence, this study was carried out to investigate the occurrence, and the associated clinical and laboratory findings of neonatal isoerythrolysis in Arabian horses of Khouzestan.

Results

Clinical signs and characteristics

The studied arabian horses were 11 male and 9 female neonatal foals which ranged in age between 6 hours to 6 days. The dams were between 4 to 16 years old and had 0 to 12 previous deliveries. All foals were properly fed with colostrum in the first day of birth and no abnormal signs were recorded in clinical examinations except in one (Table 1). The latter mentioned was a 3 day old male foal which was referred to the Veterinary Hospital, Shahid Chamran University of Ahvaz, Iran, with the history of acute lethargy and icterus for the last few hours while it was otherwise born and fed normally in the first 2 days of birth. The mare had one previous normal pregnancy with no NI history in the offspring. The clinical examination revealed severely icteric mucous membranes (Figure 1), hemoglobinuria, hypothermia with increased heart and respiratory rates. Neonatal isoerythrolysis causing hemolytic anemia was suspected according to the intensity of anemia and icterus and absence of prior illness in the foal, the mare’s breeding history, and exclusion of differential diagnoses. Additionally, the diagnosis was supported by the incompatibility on cross-matching.

Therapeutic measures including fluid therapy and blood transfusion were considered. However, the foal did not survive for transfusion to be performed.

Cross-match test

A definitive diagnosis of NI was made in one foal based on the erythrocyte agglutination in exposure to maternal serum with the titer of 1:128.
Table 1. Characteristics of studied foals.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Foal age (days)</th>
<th>Dam age (years)</th>
<th>Dam Parity (except the present foal)</th>
<th>Agglutination titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>1:128</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>4</td>
<td>8</td>
<td>3</td>
<td>1:4</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>6</td>
<td>11</td>
<td>7</td>
<td>1:4</td>
</tr>
<tr>
<td>4</td>
<td>Female</td>
<td>6 hours</td>
<td>10</td>
<td>3</td>
<td>1:2</td>
</tr>
<tr>
<td>5</td>
<td>Female</td>
<td>1 days</td>
<td>13</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>3 days</td>
<td>4</td>
<td>0</td>
<td>1:2</td>
</tr>
<tr>
<td>7</td>
<td>Male</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>1:8</td>
</tr>
<tr>
<td>9</td>
<td>Female</td>
<td>2 days</td>
<td>4</td>
<td>0</td>
<td>1:2</td>
</tr>
<tr>
<td>10</td>
<td>Male</td>
<td>4</td>
<td>8</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>Female</td>
<td>3 days</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>Female</td>
<td>3 days</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>Female</td>
<td>3 days</td>
<td>8</td>
<td>4</td>
<td>1:4</td>
</tr>
<tr>
<td>14</td>
<td>Male</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>1:2</td>
</tr>
<tr>
<td>15</td>
<td>Male</td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>Male</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>1:2</td>
</tr>
<tr>
<td>17</td>
<td>Male</td>
<td>2</td>
<td>16</td>
<td>12</td>
<td>1:2</td>
</tr>
<tr>
<td>18</td>
<td>Male</td>
<td>2</td>
<td>7</td>
<td>3</td>
<td>1:8</td>
</tr>
<tr>
<td>19</td>
<td>Female</td>
<td>1 days</td>
<td>10</td>
<td>5</td>
<td>1:4</td>
</tr>
<tr>
<td>20</td>
<td>Male</td>
<td>16 hours</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

(Table 1). Based on titration results, foals were devided into five groups to compare hematologic and biochemical data. However, it should be noted that as there was only one NI case found in this study, the data obtained was impossible to be statistically compared to other groups.

**Hematologic assessment**

The foal with hemolytic anemia showed a major decline in HCT, Hb and RBC, despite no significant difference in erythrocyte parameters in other foals (Table 2). Erythrocyte morphologic changes in the anemic foal included marked anisocytosis, mild polychromasia and echinocytosis, and a low number of metarubricytes.

Leukocyte total and differential counts did not reveal any significant differences between groups (Table 2). However total leukocyte and neutrophil count was considerably higher in the NI foal (titre of 1:128).

Erythrocyte indices and platelet count did not differ between groups including the NI foal.

**Biochemical assessment**

The lowest amount of serum glucose and protein was observed in NI foal while there was no significant difference in oth-
Table 2.
Hematologic results as mean ± SE in various neonatal foal groups based on erythrocyte agglutination test results.

<table>
<thead>
<tr>
<th>Erythrocyte agglutination titer</th>
<th>0 (n=7)</th>
<th>1:2 (n=6)</th>
<th>1:4 (n=4)</th>
<th>1:8 (n=2)</th>
<th>1:128 (n=1)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (×10^3/μL)</td>
<td>7.08 ± 1.45</td>
<td>4.18 ± 0.75</td>
<td>6.00 ± 1.91</td>
<td>5.65 ± 1.37</td>
<td>14.9</td>
<td>0.264</td>
</tr>
<tr>
<td>Lymph (×10^3/μL)</td>
<td>1.81 ± 0.49</td>
<td>0.9 ± 0.13</td>
<td>1.1 ± 0.11</td>
<td>0.9 ± 0.0</td>
<td>1.3</td>
<td>0.173</td>
</tr>
<tr>
<td>Mono (×10^3/μL)</td>
<td>0.36 ± 0.07</td>
<td>0.23 ± 0.04</td>
<td>0.23 ± 0.06</td>
<td>0.25 ± 0.1</td>
<td>0.7</td>
<td>0.234</td>
</tr>
<tr>
<td>Neut (×10^3/μL)</td>
<td>4.9 ± 1.04</td>
<td>2.98 ± 0.61</td>
<td>4.66 ± 1.77</td>
<td>4.5 ± 1.27</td>
<td>12.9</td>
<td>0.341</td>
</tr>
<tr>
<td>Eos (×10^3/μL)</td>
<td>0.0 ± 0.0</td>
<td>0.04 ± 0.03</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0</td>
<td>0.344</td>
</tr>
<tr>
<td>RBC (×10^6/μL)</td>
<td>10.8 ± 0.62</td>
<td>10.9 ± 0.56</td>
<td>10.44 ± 1.07</td>
<td>10.66 ± 1.29</td>
<td>2.33</td>
<td>0.911</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>16.41 ± 0.61</td>
<td>14 ± 1.76</td>
<td>14.83 ± 0.85</td>
<td>13.65 ± 2.01</td>
<td>4</td>
<td>0.403</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>47.18 ± 2.05</td>
<td>46.01 ± 1.88</td>
<td>46.73 ± 5.23</td>
<td>46.35 ± 7.67</td>
<td>10.8</td>
<td>0.938</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>45.71 ± 1.62</td>
<td>42.36 ± 0.74</td>
<td>44.83 ± 1.6</td>
<td>43.4 ± 1.69</td>
<td>46.4</td>
<td>0.193</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>12.25 ± 0.4</td>
<td>14.36 ± 0.24</td>
<td>14.33 ± 0.89</td>
<td>12.7 ± 0.35</td>
<td>17.1</td>
<td>0.263</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>33.51 ± 0.34</td>
<td>34.01 ± 0.34</td>
<td>32.06 ± 1.68</td>
<td>29.3 ± 0.35</td>
<td>37</td>
<td>0.190</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>17.28 ± 0.37</td>
<td>17.46 ± 0.13</td>
<td>16.56 ± 0.26</td>
<td>17.1 ± 0.00</td>
<td>16</td>
<td>0.184</td>
</tr>
<tr>
<td>Plt (×10^3/μL)</td>
<td>204.83 ± 15.01</td>
<td>166.83 ± 27.76</td>
<td>139 ± 50.71</td>
<td>184 ± 25.45</td>
<td>112</td>
<td>0.316</td>
</tr>
</tbody>
</table>

There were no significant difference in hematologic parameters between groups (p ≥ 0.05).

Table 3.
Biochemical results as mean ± SE in various neonatal foal groups based on erythrocyte agglutination test results.

<table>
<thead>
<tr>
<th>Erythrocyte agglutination titer</th>
<th>0 (n=7)</th>
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<th>1:4 (n=4)</th>
<th>1:8 (n=2)</th>
<th>1:128 (n=1)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>37 ± 7.18</td>
<td>48.83 ± 7.29</td>
<td>35.33 ± 7.53</td>
<td>22.5 ± 2.5</td>
<td>38</td>
<td>0.681</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.53 ± 0.2</td>
<td>1.64 ± 0.3</td>
<td>1.46 ± 0.23</td>
<td>1.55 ± 0.19</td>
<td>1.2</td>
<td>0.981</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>112 ± 20.39</td>
<td>129.33 ± 33.69</td>
<td>163.67 ± 25.11</td>
<td>62 ± 19</td>
<td>40</td>
<td>0.162</td>
</tr>
<tr>
<td>Protein (g/dl)</td>
<td>5.6 ± 0.43</td>
<td>5.5 ± 0.36</td>
<td>5.26 ± 0.5</td>
<td>6.4 ± 0.3</td>
<td>4.7</td>
<td>0.858</td>
</tr>
<tr>
<td>Alb (g/dl)</td>
<td>3.57 ± 0.15</td>
<td>3.56 ± 0.22</td>
<td>3.53 ± 0.26</td>
<td>4.25 ± 0.15</td>
<td>3.7</td>
<td>0.992</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>115.14 ± 19.55</td>
<td>129.33 ± 27.31</td>
<td>97 ± 8.73</td>
<td>95.00 ± 92.00</td>
<td>304</td>
<td>0.725</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>4045.7 ± 665.38</td>
<td>4509.3 ± 756.54</td>
<td>2830.3 ± 1302.8</td>
<td>3982 ± 700.0</td>
<td>2632</td>
<td>0.531</td>
</tr>
<tr>
<td>GGT (U/l)</td>
<td>34.00 ± 11.81</td>
<td>35.00 ± 15.00</td>
<td>30.00 ± 9.7</td>
<td>38.50 ± 8.04</td>
<td>55</td>
<td>0.601</td>
</tr>
<tr>
<td>Bilirubin total (mg/dl)</td>
<td>2.92 ± 0.28</td>
<td>2.77 ± 0.29</td>
<td>3.19 ± 0.98</td>
<td>2.39 ± 0.38</td>
<td>26.49</td>
<td>0.759</td>
</tr>
<tr>
<td>Bilirubin direct (mg/dl)</td>
<td>0.35 ± 0.021</td>
<td>0.35 ± 0.02</td>
<td>0.31 ± 0.04</td>
<td>0.46 ± 0.005</td>
<td>12.55</td>
<td>0.612</td>
</tr>
</tbody>
</table>

There were no significant difference in serum biochemical parameters between groups (p ≥ 0.05).

er groups (Table 3). Serum total and direct bilirubin levels in the affected foal were more than 10 times the mean values in various groups along with a relative increase in AST and GGT activity (Table 3).

Discussion

This study was performed to investigate the occurrence of neonatal isoerythrolysis in Arabian horses of Khouzestan, South-West Iran. Out of 20 newborn foals studied in the region, one case of acute NI was found. The symptoms were in accordance with the previously reported NI cases in other breeds which most obviously includes icterus, tachycardia and tachypnea and eventually death [2,3,11,12]. Many of the clinical signs observed are a result of the decrease in oxygen-carrying capacity of blood [13].

The dam, gave birth to the NI foal, was 6 years old with one previous foaling. Although it did not seem to be any correlation between parity and the foal hemolytic disease, yet no positive case was found in primiparous mares here. However, NI has already been reported in foals born to primiparous mares with no history of previous blood transfusion [14] which might be attributed to transplacental sensitization of the mare to fetal erythrocytes in early pregnancy.
Neonatal isoerythrolysis was reported in various horse breeds. However, there was no previous documented report of NI in Iran and it was recorded for the first time in Arabian horses of Khouzestan through the present study.

In a 5 year retrospective study of clinical cases of NI in foals, the disease was recorded in American Paints, warmblood cross, Standardbred, Thoroughbreds, and Quarter Horses. There were no affected Arabian horses despite that they accounted for about 10% of total neonatal equid population at the studied hospital [2].

In another study, neonatal isoerythrolysis was reported in a 5-day-old female Standardbred foal with icterus and listlessness which was diagnosed based on agglutination in cross-match between the mare serum and foal erythrocytes. The unusual finding was the marked reticulocytosis displayed with automated and manual methods [15].

In the present investigated Arabian foals, neonatal isoerythrolysis was associated with erythrocyte agglutination with mare serum diluted up to 1:128. There were substantial leukocytosis and neutrophilia along with a major decline in hematocrit, hemoglobin concentration and erythrocyte count with a slight decrease in platelet count in the affected foal compared to healthy newborn animals. It should be noted that in other foals, the agglutination titers of 1:2 to 1:8, which were considered negative, did not accompany any significant change in leukocyte or erythrocyte parameters, as expected.

Anemia as one of the characteristics of the disease is caused by attachment of maternal alloantibodies to neonatal RBCs resulting in primarly hemolysis or hemagglutination followed by extravascular or intra-vascular hemolysis [1]. Thrombocytopenia, has been reported concurrently with NI, in horse and mule foals [2,16,17] which might be attributable to anti-platelet alloantibodies, coagulopathies due to inflammatory or hypoxic-ischemic injuries, or other causes.

Increased WBC and neutrophil counts was reported previously by Boyle et al (2005) in some of the foals with NI [2]. This neutrophilia is most possibly due to sympathoadrenal and neurohormonal responses to anemia. However, Wong et al. (2012) observed persistent neutropenia, without any detectable infection, coupled with neonatal isoerythrolysis in a 3-day-old Thoroughbred colt [14]. A positive granulocyte agglutination test with the mare’s serum along with flow cytometric analysis led to a clinical diagnosis of alloimmune neonatal neutropenia. The foal was treated successfully with prophylactic antimicrobials combined with recombinant human granulocyte colony-stimulating factor (rhG-CSF).

In biochemical analysis, bilirubin concentration (total and direct) in the NI case was about ten times higher than the rest of the foals. In contrast, serum glucose and protein levels were extremely reduced in the foal with hemolytic anemia when compared to others.

Icterus as a result of increased serum indirect bilirubin, secondary to hemolysis, is one of the key features of neonatal isoerythrolysis. However, direct hyperbilirubinemia has rarely been reported in association with NI [2] which presumably indicates hepatocellular damage due to bilirubin overload in hepatocytes and hemosiderosis in addition to anemic hypoxia in the studied hemolytic foal. Serum AST activity was consistent with these findings [18]. Uncommonly, toxic hepatopathy (from severe hemolysis) or hepatocellular necrosis (from anoxia) may result in an increase in concentrations of liver enzymes, ammonia, and bile acids [13].

A Retrospective case series study of 72 foals with NI by Polkes et al., (2008) revealed that the most common causes of death or euthanasia in these animals were development of liver failure, kernicterus, and complications related to bacterial sepsis [19]. In addition, they found that total bilirubin concentrations more than 27 mg/dL can increase the chances of developing kernicterus up to 17 times in foals with NI. In the same context, kernicterus was diagnosed at necropsy in a 5 day old Thoroughbred foal that died following a clinical history of seizure and severe icterus.

In biochemical analysis, bilirubin concentration (total and direct) in the NI case was about ten times higher than the rest of the foals. In contrast, serum glucose and protein levels were extremely reduced in the foal with hemolytic anemia when compared to others.

This study described the occurrence of neonatal isoerythrolysis in Arabian horses of Khouzestan, for the first time. The diagnosis was made based on cross-match test between mare and foal, clinical examinations and laboratory analysis. The symptoms of the disease were icterus, hemoglobinuria, tachycardia and tachypnea while severe anemia, leukocytosis and hyperbilirubinemia were the most significant laboratory signs. These findings can be beneficial in the establishment of preventive measures in Arabian horse breeding industry in the region as well as improving therapeutic methods.

Materials and Methods

Animals and sample collection
This study was performed on Arabian horses in Khouzestan province, a subtropical area located in the South-West of Iran. A total of 20 neonatal foals under one week of age and their dams were included in this study from January 2017 through December 2017. All animals were used after institutional approval of the Animal Handling Committee of Shahid Chamran University of Ahvaz. Each foal’s signalments including age, sex, colostrum ingestion in the first 24 hour of birth, and the number of previous deliveries (parity) of the dam was recorded. In addition, the foals were clinically examined regarding the condition of mucous membranes, heart and respiratory rates, body temperature and the presence of hemoglobinuria and neurological symptoms.

Blood samples were collected from the jugular vein of foals and mares into tubes with anticoagulant (EDTA) for hematologic and agglutination tests and without anticoagulant for serum separation and biochemical analysis.

Cross-match test

A cross-match between the mare serum and foal erythrocytes was performed in order to detect the presence of anti-foal erythrocyte antibodies in the mare serum. Briefly, EDTA-anticoagulated foal blood samples were washed 3 times with phosphate buffered saline (PBS) and after the final washing, a 2% suspension of red blood cells in PBS was prepared.

The agglutination test was performed in a 96-well microplate. Fifty μl of the mare serum was added to 50 μl of PBS in the first well of the row, and then increasing dilutions of 1:2 antisera in PBS were prepared through 1:4096. Afterwards, 50 μl of foal washed RBCs suspension was added to each well and incubated at 37°C for 30 min and then for 30 min at room temperature (25°C). The additional 30 min at room temperature was conducted to permit RBCs to settle and agglutination patterns to form.

Wells were recorded as negative if they contained a button or a button with a more extensive spread of RBCs that would disperse when the microtiter plate was slanted; positive wells exhibited mat formation that did not disperse when slanted, and the titer of positivity was recorded. The test was considered positive if agglutination occurred at 1:16 antibody dilutions.

Hematologic assessment

Hematological parameters including erythrocyte count (RBC), hematocrit (HCT), hemoglobin concentration (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and leukocyte count (WBC) were determined by the BC-2800 Vet hematology analyzer (Mindray, China). Differential leukocyte counts and erythrocyte morphology were also evaluated in microscopic examination of blood smears.

Biochemical assessment

Serum biochemical parameters including total protein, albumin, glucose, urea, creatinine, total and direct bilirubin concentration and AST, ALP and GGT activities were assessed with a biochemistry autoanalyzer (BT-1500, Biotechnica, Italy) using Parsazmun kits (Iran).

Statistical analysis

ANOVA and Tukey’s Post Hoc tests were employed to compare laboratory-obtained values between groups using SPSS software Version 16 (SPSS Inc., Chicago, IL, USA). All values were expressed as mean and standard error (SE) and p < 0.05 was considered as statistically significant.

Aknowledgements

The authors wish to express their gratitude to research council of Shahid Chamran University of Ahvaz for their financial support. The authors would like to thank all veterinarians and technicians in the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz who helped in sample collection.

Author contributions

Conceived and designed the experiments: S.M.J., M.R.J., A.G.M. Performed the experiments: S.M.J., A.G.M., M.M.Z. Wrote the paper: S.M.J.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Molecular epidemiology of *Campylobacter Fetus* in aborted fetuses of Baluchi sheep in Sistan region

Ebrahim Hossein Abadi, Dariush Saadati, Mohsen Najimi, Mehdi Hassanpour

**Keywords**

abortion, *Campylobacter*, sheep, Sistan, PCR

**Abstract**

*Campylobacter* is one of the main bacterial causes of ewe abortion throughout the world. *Campylobacter* infections are now considered as zoonoses. The objective of this study was an investigation of *Campylobacter fetus* prevalence among aborted ovine fetuses in the Sistan region (north of Sistan and Baluchestan province). In the present study, spleen and abomasum content samples were obtained from 78 aborted lambs of Baluchi sheep. The samples were examined for *campylobacter* contamination using PCR method. The overall prevalence of *campylobacter* infection was 7.7%. The prevalence of infection in fetuses aged three months and under were significantly higher than that in fetuses older than three months. The result of this study showed that *C. fetus* should be considered as one of the infectious causes of abortion among sheep flocks in Sistan region.

**Abbreviations**

PCR: polymerase chain reaction  
*C. fetus*: *Campylobacter fetus*
Introduction

Campylobacter is one of the main bacterial causes of ovine abortion in the world. This agent is one of the main causes of sheep abortion in New Zealand [1] and prevalence rate of abortion in flocks which are infected with campylobacter in America, is 23.2% on average [2]. Campylobacteriosis is a highly contagious infection. The fetus, placenta, birth fluids, vaginal discharge, and feces from infected ewe are all sources of infection. If the water or forage become contaminated with these materials, the infection can rapidly spread in the flocks [3]. Several investigations in Iran indicate that Brucellosis, Chlamydiosis, Mycoplasmosis and Campylobacteriosis are the major causes of abortion in sheep in the country [4-6].

Campylobacter fetus is now considered as a zoonotic disease. Products from cattle and sheep are suspected as sources for human infections. Infection of human with C. fetus mostly begins with the oral ingestion of the bacterium followed by intestinal colonization. Some of colonized individuals induce diarrhea. Occasionally, C. fetus causes severe systemic infections. Systematic infections mainly affect elderly and immunocompromised individuals [7].

Campylobacteriosis in sheep can be characterized with different clinical forms including, abortion, stillbirths, and birth of weak lambs [8]. This infection usually occurs in flocks by the introduction of new carrier animals, and susceptible ewes may acquire the infection by ingestion of contaminated feed and water [9, 10]. Keeping sheep in contact with other domestic animals such as goats, camel and poultry that are usually subjected to an inferior quality or absence of veterinary care will encourage the risk of transmission of the infectious agent within the flock [10].

However, recent studies show that abortion caused by Campylobacter jejuni is on the rise, but C. fetus is considered as the main cause of ovine abortion among campylobacter species [3, 11, 12].

Rapid diagnosis of an abortion agent has a great importance in prevention and control of the disease [13]. Old diagnostic methods of campylobacteriosis are time-consuming, partly difficult and are not always accurate. Thus, molecular methods such as PCR are welcomed in the recent years, particularly in research studies [14].

One of the major economic problems of sheep breeding in the Sistan region is abortion. Proper management of Campylobacter infection plays an important role in the prevention and control of sheep abortion [10]. This study was conducted to investigate the presence of C. fetus in the Sistan region (northern of Sistan and Baluchestan province).

Results

Among 78 aborted fetuses, 6 cases (7.7%) (95% CI: 2.9% - 16.0%) were infected with campylobacter. Figure 1 shows the results of electrophoresis in contaminated samples with C. fetus.

Prevalence of infection in fetuses under three months of age was statistically more than that in fetuses over three months of age. The association between other independent variables and campylobacter infection was not statistically significant (Table 1).

Among 78 aborted fetuses, 2 spleens (3%) and 4 abomasum (5%) were contaminated with C. fetus. MacNemar test shows that the differences of contamination between spleen and abomasum were not statistically significant.

Discussion

In the present study, C. fetus was isolated from 7.69% of fetuses. Prevalence of this bacterial pathogen in aborted fetuses in Fars province in 2005 was 7.5% [4] and the prevalence in Hamedan province in 2010 was 1.4% [15]. In a study conducted in Turkey in 2010, the prevalence of infection with C. fetus in aborted fetuses was 6.6% [16]. In a study by Agerholm and colleagues in Denmark 24 samples from the stomach contents of aborted fetuses were examined by culture method in which one sample was positive [17].

In the present study, PCR method was used for detecting and identifying campylobacter. PCR method is a rapid and worthwhile diagnostic test [9]. Tuzcu et al. compared immunohistochemistry, microbiology, pathology, and PCR methods for diagnosis of campylobacteriosis in the aborted bovine fetuses. Their re-
is an appropriate place for isolation of \textit{C. fetus} \cite{13,18}. The gallbladder sample is also useful for the detection of \textit{campylobacter} \cite{19}. Furthermore \textit{campylobacter} can be isolated from the placenta and with lower success from the liver and lung samples of an aborted fetus \cite{2}. However, the absence of \textit{Campylobacter} in both organs of spleen and abomasum show that taking several samples from different organs is necessary to detect infection with \textit{C. fetus}.

According to the results of the present study, the prevalence of infection with \textit{C. fetus} in fetuses three months and under was significantly higher than that in older fetuses, however, most of the other studies reported that abortion due to \textit{C. fetus} often occurs in late pregnancy \cite{7,20}. More investigations in this field in Sistan region would be necessary to find out the causes.

The result of this study showed that \textit{PCR} is the most accurate method for identifying this infectious agent \cite{16}. As the PCR is based on DNA detection, it is a more accurate method in comparison with other methods such as microbiology, immunohistochemistry, pathology and serology for the detection and identification of most infectious agents.

In the present study, 2 spleens (3\%) and 4 abomasal content samples (5\%) were infected with \textit{C. fetus}. The difference between prevalence of \textit{campylobacter} in spleen and abomasum was not statistically significant. Other studies show that stomach content

### Table 1.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>levels</th>
<th>No. of tested fetuses</th>
<th>No. of positive fetuses</th>
<th>Prevalence</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of livestock</td>
<td>Zahak</td>
<td>12</td>
<td>0</td>
<td>0%</td>
<td>0.180</td>
</tr>
<tr>
<td></td>
<td>Hirmand</td>
<td>12</td>
<td>0</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nimrooz</td>
<td>9</td>
<td>1</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zabol</td>
<td>38</td>
<td>5</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hamoon</td>
<td>6</td>
<td>0</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

| History of abortion   | Yes    | 3                     | 0                       | 0\%        | 0.784        |
|                       | No     | 75                    | 6                       | 8\%        |              |

| Sex of fetus          | Male   | 38                    | 1                       | 3\%        | 0.112        |
|                       | Female | 40                    | 5                       | 13\%       |              |

| Age of fetus          | \( \leq 3 \) Month | 12                    | 3                       | 25\%       | 0.044        |
|                       | 4-5 Month     | 66                    | 3                       | 5\%        |              |

| Age of ewe            | \( \leq 2 \) years | 26                    | 3                       | 12\%       |              |
|                       | 2-5 years     | 41                    | 3                       | 7\%        | 0.230        |
|                       | 5 years\( \geq \) | 11                    | 0                       | 0\%        |              |

| Parity of ewe         | First        | 25                    | 3                       | 12\%       |              |
|                       | Second       | 23                    | 0                       | 0\%        |              |
|                       | Third        | 18                    | 3                       | 17\%       | 0.602        |
|                       | Forth and above | 12                | 0                       | 0\%        |              |

### Table 2.

Characteristics of primers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
<th>Product length</th>
<th>Microorganism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA</td>
<td>F: TTTGTTAGGGAAGAACCATG</td>
<td>265 bp</td>
<td>Campylobacter fetus</td>
<td>Saleh et al., 2013 [21]</td>
</tr>
<tr>
<td></td>
<td>R: CGCAATGGGTATTCCTGGT</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
sterile blade before DNA extraction. Genomic DNA was extracted from spleen and abomasal content samples using DNP TM Kit, High yield DNA Purification Kit (CinnaGen., Tehran, Iran), according to the manufacturer's instructions. DNA quality was measured spectrophotometrically and low concentration samples (lower than 100 ng/μL) were excluded from further analysis.

Primers used were according to a previous study [21] (Table 2). PCR reactions were performed in 15 μl volume (including forward and reverse primers (10 pmol/μl), master mix (containing 3 mM MgCl2, 0.4 mM dNTPs, 0.2 units/μl Ampliqon Taq DNA polymerase), and isolated DNA). Parameters used were initial denaturation at 94°C for 4 minutes, afterward denaturing at 94°C for 45 second, annealing at 64°C for 1 minutes, extension at 72°C for 1 minute and a final extension of 72°C for 10 minutes. Then, PCR products were run on 2% agarose gel electrophoresis (80 v and 220 mA for 75 minutes), followed by staining with ethidium bromide and visualized under UV (Cambridge gel documentation). Positive and negative controls were included in all reactions.

Correlations between independent variables (location of livestock, age and sex of the fetus, abortion history, age and parity of the aborted ewe) and dependent variable (fetus contamination with campylobacter) were investigated with Chi-square and Fisher exact tests, Age and parity of the ewe were considered as ordinal variables, so their correlation with fetus contamination were investigated with linear by linear Chi-square.

Contamination rates of spleen and abomasum to campylobacter were compared with McNemar test. All statistical analysis was performed using SPSS v.18.0, with significance level of 5% (IBM Corp., Armonk, NY, USA).

Acknowledgments

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Author contributions

Conceived and designed the experiments: EHA, MH, MN, DS. Collected the foetuses: EHA. Performed autopsy of the foetuses: EHA. Performed the experiments: EHA, MN. Analyzed the data: DS. Provided research space and equipment: MN. Wrote the paper: EHA and DS.

Conflict of interest

None of the authors have any conflict of interest to declare.

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Campylobacter in ovine aborted fetuses in Sistan


In vitro evaluation of acaricidal activity of aqueous ozone against *Dermanyssus gallinae*

Hamid Dehghan Banadakei, Ali Moshaverinia, Mohammad Hossein Haddad Khodaparast, Gholam Ali Kalidari

**Keywords**
Acaricide, aqueous ozone, *Dermanyssus gallinae*, laboratory conditions

**Abstract**
The aim of this study was to investigate the in vitro acaricidal effect of aqueous ozone against *Dermanyssus gallinae* (Acaric: Dermanyssidae) under laboratory conditions. For this purpose, aqueous ozone at concentrations of 1, 2, 3, 4, 5 and 10 ppm were prepared, and five replicate experiments were carried out using 400 µl of each concentration sprayed on every treatment batch of mites (plus a distilled water control). The mortality rate of mites in treatment and control groups were assessed 24 h post exposure. The mortality rate obtained by concentrations of 4, 5, and 10 ppm were significantly different from the control group (p < 0.05). The highest mortality rate (63.99%) was observed at concentration of 10 ppm. In this study, aqueous ozone showed a dose-dependent acaricidal potency against *D. gallinae*.

**Abbreviations**
*ppm*: parts per million

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**Introduction**

*Dermanyssus gallinae* negatively affects chickens’ health via biting and sucking blood which consequently can cause economic losses due to a decrease in egg production, down-grade eggs, increase in mortality of laying hens and transmitting some pathogenic agents [1].

Extensive and repeated usage of conventional chemical compounds in commercial poultry farms has led to the development of acaricide resistance in *D. gallinae* [2, 3]. Acaricide resistance and increased demand for organic foods have motivated researchers to investigate novel ways of controlling this ectoparasite, especially in commercial poultry houses [4].

Ozone is a strong oxidant. It is an allotrope of oxygen and consists of three oxygen atoms. It is an unstable molecule with short half-life and readily degrades into O$_2$ and a free oxygen atom. This atom disrupts and kills microorganisms through reaction with the cell membrane and cellular components [5]. Ozone is commonly generated using an electrical discharge. In recent years, the efficacy of ozone as a means of controlling post-harvest grain pests has been evaluated. Insecticidal effects of gaseous ozone against the confused flour beetle (*Tribolium confusum*), the red flour beetle (*Tribolium castaneum*), maize weevils (*Sitophilus zeamais*) and Indian meal moths (*Plodia interpunctella*) have been investigated and ozone was found to be efficacious against stored product insects under both laboratory and field conditions [6-8].

The aim of this study was to evaluate the acaricidal activity of aqueous ozone specifically against *D. gallinae* under laboratory conditions.

**Results**

The examined groups of mites exposed to aqueous ozone at concentrations of 1, 2, 3, 4, 5 and 10 ppm showed 13.13, 25.99, 36.66, 47.33, 53.99 and 63.99% mortality, respectively. The mortality rates were dependent on ozone concentration, with ascending mortality rate as concentration increased (*p* < 0.05). The mortality rates at 4, 5 and 10 ppm concentrations of aqueous ozone were significantly different than the control group (*p* < 0.05). The highest mortality rate (63.99%) was observed at concentration of 10 ppm (Figure 1).

**Discussion**

Huge economic losses imposed by *D. gallinae* infestation across the world and restriction in the use of conventional acaricides, indicates that searching for new control strategies is an important necessity. Because ozone acts as a strong oxidizer, it is highly toxic to living organisms such as microorganisms, fungi, insects and mites [9, 10]. Ozone is thought to kill organisms by oxidation of cell membrane and cellular components such as enzymes, proteins, fatty acids, it can also destruct and damage nucleic acids [11, 12].

Whilst working with aqueous ozone is much safer than gaseous ozone and neither hazardous to skin nor to eyes, a few researches have been conducted on acaricidal/insecticidal activity of ozonized water [13]. Besides these advantages, reaction between ozone and water also results in the formation of highly reactive radicals such as *OH, *O$_2^-$, *HO$_2^-$ and *O$_3^-$ which can cause fatal damage to respiratory system of insects and acarians [12]. All ozone derivative radicals can affect adversely cellular membranes and collapse DNA (deoxyribonucleic acid) structure of affected cells, among them hydroxyl free radical is much more powerful [12, 14]. Despite the lack of documented researches about various practical uses of aqueous ozone in livestock and poultry industry, it is already in use in dairy farms and relevant industries [15]. Also, a portable ozone mist system for the pest control in farms has been developed [12].

In this preliminary study, the acaricidal potency of aqueous ozone against *D. gallinae* was demonstrated, with an average mortality of 64% at ozone concentration of 10 ppm. However, to the best of our knowledge, no study has yet investigated the effect of ozone against poultry red mites. Several factors influence ozone effectiveness against a living organism including method of application, concentration, exposure time, type of organism, life stage of organism, temperature and relative humidity [16, 17]. Exposure of *Plodia interpunctella* to 500 ppm gaseous ozone for 60 minutes resulted in 100% mortality [18]. Niakausari et al. (2010) reported complete mortality of adult *Phoe**

**Figure 1.**
Mean mortality (%) (± SE) of *Dermanyssus gallinae* exposed to various aqueous ozone concentrations (ppm). Means designated by different letters are significantly different (*p* < 0.05).
*nix dactylifera* when exposed to gaseous ozone at concentrations more than 2000 ppm for 2 h [19]. In the present study, based on statistical analysis, increasing in ozone concentration can result in more mortality of *D. gallinae*, however; producing and applying ozonized water with more than 10 ppm concentration due to our technical limitations and safety concerns was not possible. Niakausari et al. (2010) also found that ozone at lower concentrations required a much longer exposure time to be effective [19]. Due to the unstable nature of aqueous ozone, we tried to increase the exposure time by increasing ozone half-life time. Half-life time of ozone is notably affected by temperature and researchers have found that lower temperatures optimize the half-life of aqueous ozone [20]. At 20°C and pH 7, the half-life of ozone in potable tap water is about 24 minutes and in our study drinking tap water at 10°C was used to achieve longer ozone half-life time.

In this study, aqueous ozone showed a dose-dependent acaricidal potency against *D. gallinae*. These findings cannot be extrapolated directly to decreasing the mite population in infested poultry farms and increasing productivity as a consequence of spraying aqueous ozone. Further researches should be done to determine the concentration of aqueous ozone with the highest acaricidal activity, and the least probable adverse effects of aqueous ozone on birds’ health in poultry farms at rear ing and egg-production.

**Materials and methods**

**Mite collection and preservation**

*D. gallinae* were collected from a naturally infested caged laying poultry farm and were kept at 22°C with a 16:8 light - dark cycle.

**Ozone generation**

Ozone was produced using a laboratory corona discharge ozone generator (Ozone Tajhiz Co. Mashhad, Iran). The Maximum concentration of 143 g/m³ was obtained at oxygen flow rate of 1.5 L/min. Gaseous ozone was injected into a water column for producing aqueous ozone. Ozone concentration in the column was determined using a colorimetric kit (CHECK kit® Comparator ozone, The Trinometer Hg, England). Ozonized water through a valve located at the bottom of water column was poured into a glass beaker for determining the ozone concentration, then the exposure time by increasing ozone half-life time. Half-life time of ozone is notably affected by temperature and researchers have found that lower temperatures optimize the half-life of aqueous ozone [20]. At 20°C and pH 7, the half-life of ozone in potable tap water is about 24 minutes and in our study drinking tap water at 10°C was used to achieve longer ozone half-life time.

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**Bioassay**

For this study, six treatment groups and one control group were set up. A petri dish containing over 250 mites was placed in a freezer at 0°C for five minutes to immobilize them. Seven batch of mites (each batch contained 20 mites) were separately transferred to a glass petri dish (90 mm diameter and 15 mm depth) lined with Whatman filter paper by an aspirator. Different aqueous ozone concentrations of 1, 2, 3, 4, 5 and 10 ppm were prepared and 400 μl of desired concentration was sprayed on each treatment group, using a handheld sprayer. The control group was subjected to distilled water with the same procedure. Each petri dish was sealed using parafilm before the lid was placed on top. The plates were incubated at 22°C for 24 h. Finally, mite mortality was examined under a dissecting microscope, where a mite was considered dead if it did not show any sign of movement when it was agitated with an entomological pin. The bioassay was repeated five times.

**Statistical analysis**

All data were analyzed using SPSS ver. 22 for Windows (SPSS Inc., Chicago, Illinois) and one-way analysis of variance (ANOVA) was used for comparing mortality rate. Pearson correlation test was used to determine the relationship between ozone concentration and mite mortality. Mortality rates were adjusted using Abbott’s formula. *p* values less than 0.05 were regarded statistically significant.

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**Author contributions**

Conceived and designed the experiments: AM. Performed the experiments: HDB, AM, MHHK, GAK. Analyzed the data: AM. Wrote the paper: AM.

**Conflict of interest**

The authors declare that there is no conflict of interests.

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مروری بر اثرات قلبی گیاهان سمی ایران

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گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید کردکوی، شهرکرد، ایران

چکیده:
این مقاله مروری بر اثرات سمی گیاهان ایران است. در بین گونه های متنوع گیاهان ایران، تعداد زیادی حاوی ترکیبات شیمیایی هستند که به‌کار دام ها و انسان سمی هستند. در بین این گیاهان سمی، گیاهان کاردیوتوکسیک به علت طبیعت حاد مسمومیت ناشی از آنها و مرگ و میر موارد مسمومیت از اهمیت خاصی برخوردار هستند. از گیاهان خانواده نریوم Nerium oleander، N. indicum، Thevetia peruviana آپوسیناسه، انواع خرزهره شامل Taxus با جمله اولاندروزد و توتین A-C قلبی از جمله اولاندروزد و توتین می باشند. گیاهان خانواده تاکسیس، انواع سرخه دار شامل A-C قلبی از جمله اولاندروزد و توتین می باشند. گیاهان خانواده تاکسیس، انواع سرخه دار شامل A-C قلبی از جمله اولاندروزد و توتین می باشند. گیاهان خانواده تاکسیس، انواع سرخه دار شامل A-C قلبی از جمله اولاندروزد و T. brevifolia, baccata بوده که نگاه به ارزش میزان کلسیم داخل سلولی شده و بسته به دز، اثرات انرژی و با استفاده از الکتریکی قلبی دیده می شود. تاکسین B، کلسیم بالابر هیپکس در سرخه دارها با کاهش کانال های کلود و کلسیم به ویژه در میوپلیاس ها دچار اختلال در سیستم های قلب می شود. طیف متغیری از آنتی‌زیم های قلبی در موارد مسمومیت حاد گیاهان فویق دیده می شود که باعث نارسایی حاد قلبی و مرگ می شود. در این موارد، یافته های بعد از مرگ جنبه تشخیصی ندارند و آنالیز سیم‌شناختی محتوای دستگاه‌های گوارش و مایعات بدن برای مشخص کردن حضور الکتریکی قلبی با تاکسین ها مورد استفاده قرار می‌گیرد.

کلید واژه‌ها:
سمومیت با خرزهره، مسمومیت با سرخه دارها، الکتریکی قلبی، تاکسین ها، آنتی‌زیم قلبی
ساخت و ارزیابی آزمایشگاهی فیلم های بر پایه کیتوزان برای دارو رسانی آهسته رهش انروفلوکساسین

کلید واژه ها
انروفلوکساسین، کیتوزان، بتا-گلیسرофوسفات

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2 مرکز تحقیقاتی فیزیولوژی و فارماکولوژی، دانشگاه علوم پزشکی رفسنجان، رفسنجان، ایران
3 گروه دوربینولوژی، دانشکده داروسازی، دانشگاه علوم پزشکی تهران، تهران، ایران
4 گروه میکروبیولوژی، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران

چکیده:
فرآورده های دارویی کاشتنی به طور عمده برای رهایش آهسته دارو توسعه می یابند. این مطالعه به منظور ساخت و ارزیابی فیلم های کیتوزان/ بتا-گلیسرофوسفات با اتصالات متقاطع برای دارو رسانی آهسته انروفلوکساسین صورت گرفت. دو نوع فورمولاسیون فیلم، تک لایه (F1) و سه لایه (F2)، تهیه شد. ارزیابی آزمایشگاهی رهایش دارو، مدل سازی کینتیک دارو، طیف سنجی مادون قرمز فوریه و مطالعات میکروبیولوژیک انجام شد. رهایش دارو از ساعت طول کشید. میزان رهایش به ترتیب تا F3 و F4 تا 5 ساعت ادامه داشت اما رهایش دارو از F2 به ترتیب تا 96 و 168 ساعت طول کشید. میزان رهایش تجمعی دارو برای فیلم های F3 و F4 با ترتیب 74,6 و 1,7% بیشتر از F2 و F1 بود. ناحیه مدار رشد باکتری با استفاده از نمونه های کنترل های مثبت و فیلم تک لایه، به طور معنی داری پیشتر از فیلم های سه لایه بود (P < 0,05). که نشان دهنده افزایش آهسته فیلم های چند لایه است. این یافته ها نشان می دهد که فیلم های سه لایه کیتوزان/ بتا-گلیسرофوسفات می توانند برای دارو رسانی انروفلوکساسین برای یک دوره طولانی موثر باشند.

کلید وازه ها:
انروفلوکساسین، کیتوزان، بتا-گلیسرофوسفات، آهسته رهش، فیلم سه لایه
اثر اسانس 4°C ذخیره سازی بر منی خروس در Zataria multiflora

فهیمه گرمی نژاد، محمد روستائی علی مهر
گروه علوم دامی، دانشکده کشاورزی، دانشگاه گیلان، رشت، ایران

چکیده:
آزمایشی به منظور بررسی اثر اسانس آویشن شیرازی بر ذخیره سازی اسپرم خروس در 4°C انجام شد. جمع آوری منی از 15 خروس بالغ 2 بار در هفته برای 4 نوبت انجام شد. انزال ها در هر نوبت بعد از تجمع به 7 قسمت تقسیم شد. مقادیر صفر، 50، 100، 200، 400 و 1000 نانوگرم در میلی لیتر اسانس آویشن شیرازی به هر قسمت اضافه شد. سپس نمونه ها تا 4°C سرد شدند و به مدت 22 ساعت در این دما نگهداری شد. در زمان های صفر، 36، 72، 108 و 168 ساعت ذخیره سازی، تحرک، زنده مانی (رنگ آمیزی هوخست 23258 و سلامت غشای پلاسمایی اسپرم ارزیابی شد. در زمان 48 ساعت تعبین پراکسیداسیون، غلظت مالون دی آلدهید (MDA) در 4% اسپرم انداره گیری شد. نتایج نشان داد که بیشترین تحرک پیش رونده (4/32) به ماهی (31/28%) و سلامت غشای پلاسمایی اسپرم (88/51%) در سطح 200 نانوگرم در میلی لیتر مشاهده شد. کمترین تحرک پیش رونده اسپرم (1/31) به ماهی (31/28%) و سلامت غشای پلاسمایی اسپرم (88/51%) در سطح 1000 نانوگرم در میلی لیتر مشاهده شد. کمترین MDA یا 1/48 nM/mL (6/75%) رتبه بر ترتیب در مقدار 400 و 1000 نانوگرم در میلی لیتر مشاهده شد (5/00). بنابراین افزودن 200 نانوگرم در میلی لیتر اسانس آویشن شیرازی به مینی سبب بهبود ماندگاری اسپرم خروس در 4°C شد.

کلید واژه ها:
آنتی اکسیدان، آویشن شیرازی، اسپرم خروس
ارتباط بین مقادیر فروکتوز آمین، گلوکز، پروتئین تام و آلبومین سرم خون در اواخر آبستنی و ابتداي زایش در بزهای نژاد شیری سانن

روبة بورحمد، مهرداد مهری، حسام الدين سیفی

گروه علوم درمانگاهی دانشگاه فردوسی مشهد، مشهد، ایران
گروه علوم درمانگاهی و قطب علمی مطالعات سقط جنین و مرگ و میر نوزاد نشخوارکنندگان دانشگاه فردوسی مشهد، مشهد، ایران

در مطالعه حاضر تغییرات مقادیر فروکتوز آمین سرم خون در بزهای سانن در اواخر آبستنی و ابتداي زایش بررسی و ارتباط آن با غلظت های گلوکز، پروتئین تام و آلبومین سرم خون، مورد مطالعه قرار گرفت. یازده راس بز سانن در اواخر آبستنی شناخته شدند. نمونه های خون در زمان‌های مختلف در مدت ۷ روز قبل از زایش و ۳۰ روز پس از زایش آماده گردید. استفاده از روش‌های آزمایشگاهی میانگین مقدار هر مثال و همچنین نشانه‌های ویژه نشان داد. نتایج نشان داد که در اواخر آبستنی، غلظت گلوکز میانگین در بزهای سانن ۲ درصد بیشتر از غلظت گلوکز در ابتدا زایش بود. به علاوه، سیگنال‌های خاصی این گروه نشان داد که میانگین مقادیر آلبومین در اواخر آبستنی معنی‌دار بود. از نتایج مطالعه پژوهشگر به بررسی از نظر دینی و اجتماعی آبستنی پژوهشگر، می‌توان میانگین مقادیر آلبومین در اواخر آبستنی مصرف نمود. نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتایج مطالعه پیشنهاد نمود که می‌توان در نتی...
تغییرات فاکتورهای خون شناسی در بزغاله های سانن از بدو تولد تا سه ماهگی

صفوآ عبداللهی، مجید زعیمی، مهرداد مهری

چکیده:

به منظور بررسی تغییرات وابسته به سن پارامترهای خون شناسی در بزغاله های سانن از بدو تولد تا سه ماهگی، خون گیری از تعداد ۲۰ راس بزغاله که به لحاظ بالینی سالم بودند به عمل آمد. خون گیری در زمان های تا ۲۸ ساعت بعد از تولد و در روزهای انجام گردید. آزمایشات خون شناسی با استفاده از روش های مورعد بررسی قرار گرفت. نتایج این مطالعه نشان داد که تمامی متغیرهای خون شناسی به جز تعداد مونوسیت، ائوزینوفیل، و گاند با زمان اول نمونه گیری اختلاف معنی دار دارند (۵.۰۰). همچنین تمامی متغیرها به جز تعداد مونوسیت، ائوزینوفیل و گاند با زمان اول نمونه گیری اختلاف معنی دار دارند (۵.۰۰). می توان نتیجه گرفت که به منظور تفسیر دقیق نتایج خون شناسی در بزغاله های سانن نیاز به تعیین مقدار مرجع اختصاصی می باشد.

کلید واژه ها:
بزغاله، سانن، سن، نوزاد، نشخوارکننده کوچک، هماتولوژی
وقوع، خصوصیات هماتولوژیک و بیوشیمیایی سرم ایزوارینترولیز نوزادان در اسب های عرب ایران

سیده میناق جلالی ۱، محمد راضی جلالی ۱، عقیده قدردان، مهدی مشهدی ۲، مریم معتمد زرگر ۱

گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید حرم، اهواز، ایران
۲ دانش آموخته، دانشکده دامپزشکی، دانشگاه شهید حرم، اهواز، ایران

یزوارینترولیز یکی از علل اصلی کم خونی در کره های نوزاد است. با این وجود تاکنون هیچ گونه گزارش مستقلی از بروز این بیماری در اسب های عرب ایران، که بیش از همه در خوزستان پرورش می یابند، وجود ندارد. لذا این مطالعه با هدف ارزیابی وقوع یزوارینترولیز نوزادان در اسب های عرب خوزستان و بررسی تابلوی خون شناسی و بیوشیمیایی سرم در کره های مبتلا انجام گرفت. تعداد ۳۰ راس کره نوزاد با سن کمتر از یک هفته و مادران آن ها در این مطالعه مورد بررسی قرار گرفتند. در معاینات بالینی هیچ گونه علائم غیرطبیعی مشاهده نشد. تحت شرایطی که ژیست در یک کره بیمار گزارش گردید، شده که در نهایت منجر به مرگ نیز گردید. تشخیص یزوارینترولیز نوزادان براساس آگلوتیناسیون رئیسیت ها در آزمایش کراس میان سرم مادر و اریتروسیت های کره بیمار صورت گرفت که در کره یاکش به قوی‌ترین سرم مادر به شکل بافتی قابل جدایی و باعث کاهش ارگانیسم کربنیک می‌شود. این امر باعث کاهش ارگانیسم می‌شود و باعث کاهش میزان هميزان، لیپوسیتوژن و نوتروفیلی قابل توجه بود. غلظت بیلی و ثروین تام و مستقیم در کره بیمار را حدود ۱۰ برابر بیش از ساری کره های مورد بررسی بود. در مجموع در این مطالعه وقوع یزوارینترولیز نوزادان در اسب های عرب خوزستان ثبت شد که با کم خونی شدید و زردی همراه بوده و منجر به مرگ گردید. این یافته ها می تواند در ایجاد اقدامات پیشگیرانه در منطقه و تربیت درمانی باشد.
تشخیص مولکولی کمپیلوکتیک فتوس در جنین های سقط شده گوسفندان بلوچی در منطقه سیستان، ایران

ایران همی حسن آبادی؛ داریوش سعیدی، محسن نجیبی، مهدی حسین‌پور

کمپیلوکتیک یکی از عوامل باکتریایی اصلی در سقط جنین میش‌ها در سراسر دنیا است. در حال حاضر کمپیلوکتیکوز به عنوان بیماری مشترک بین انسان و حیوانات مطرح می‌شود. هدف از مطالعه اخیر بررسی شیوع آلودگی با کمپیلوکتیک فتوس در جنین های سقط شده گوسفندان در منطقه سیستان است. در این مطالعه از روش واکنش زنجیرهی پلیمراز (PCR) استفاده شد. نتایج نشان داد که شیوع آن در جنین های زیر سه ماه به طور معنی‌داری بیشتر از جنین های بالای سه ماه است. نتایج مطالعه اخیر نشان داد که کمپیلوکتیک فتوس باید به عنوان یکی از عوامل عفونتی سقط جنین در گوسفندان منطقه سیستان مورد توجه قرار گیرد.

کلید واژه‌ها: سقط جنین، کمپیلوکتیک، گوسفند، سیستان، واکنش زنجیره‌ای پلیمراز

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ارزیابی آزمایشگاهی اثر کشندگی ازن محلول در آب عليه جرب درمانیسوس گالینه

حمید دهقان یلدایی؛ علی مشاورنیا، محمد حسین حداد خداپرست، غلامعلی کلیدری

گروه پاتوبیولوژی، دانشکده دامپزشکی، دانشگاه فردوسی مشهد، مشهد، ایران

گروه صنایع مواد غذایی، دانشکده کشاورزی، دانشگاه فردوسی مشهد، مشهد، ایران

گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه فردوسی مشهد، مشهد، ایران

1397/04/11

1396/10/18

هدف از مطالعه حاضر بررسی آزمایشگاهی اثر ازن محلول در آب، روی جرب درمانیسوس گالینه بود. به این منظور
ازن محلول در آب در غلظت های 0، 1، 2، 3، 4 و 5 میکرولیتر آب مقطر، روی یک گروه 400 پی پی ام نیز به کار
گرفته شد. گروه کنترل نیز با 10 بیو پی ام اسپری شد. میزان مرگ و میر جرب ها در هر گروه
ها یک‌سان بود و درمان 24 ساعت بعد از اسپری مرد بررسی قرار گرفت. میزان مرگ و میر این گروه، به
وسیله غلظت 5 میکرولیتر آب مقطر، روی یک گروه نیز به کار گرفته شد. گروه کنترل نیز با
10 بیو پی ام اسپری شد. در این مطالعه قدرت کشندگی ازن محلول در آب بهره‌وری درمانیسوس گالینه ناشی از داده
شد. شرایط آزمایشگاهی

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IRANIAN JOURNAL OF VETERINARY SCIENCE AND TECHNOLOGY

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References for the above example:


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