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Volume 3 (2025) ISSN Editor in Chief: Edward Meinert, MA (Oxon), MSc, MBA, MPA, PhD, CEng, FBCS, EUR ING

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Original Paper

Effects of Ventral Pallidum–Nucleus Accumbens Shell Neural Pathway Modulation on Sucrose Consumption and Motivation in Female Rats: Chemogenetic Manipulation

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Abstract

Background: The neural control of food intake involves interactions between homeostatic and nonhomeostatic systems. The nucleus accumbens shell (AcbSh) and ventral pallidum (VP) play key roles in regulating ingestive behavior and project to each other. Previous studies have shown that these projections influence food consumption, with sex differences reported in the modulation of sucrose intake by VP projections.

Objective: This study aimed to investigate the effects of chemogenetic activation or inhibition of projections from the VP to the AcbSh on sucrose consumption and the motivation to work for sucrose in female rats.

Methods: Chemogenetic tools (DREADD [designer receptors exclusively activated by designer drugs]) were used to selectively activate or inhibit VP projections to the AcbSh in female Sprague-Dawley rats (Gi [inhibitory G protein] DREADD: n=11; Gq [excitatory G protein] DREADD: n=10; and no DREADD: n=12). Rats were trained on a progressive ratio operant task to assess motivation to work for sucrose. Additionally, free-access sucrose consumption tests were conducted using a 20% sucrose solution. The effects of chemogenetic modulation were analyzed using two-way ANOVA.

Results: Chemogenetic manipulation of VP projections to the AcbSh did not significantly affect the motivation to work for sucrose in the progressive ratio task ($F_{2,31}$=1.780; P=.18). However, a significant interaction between DREADD type and drug administration was observed in the sucrose consumption test. Activation of the VP-AcbSh projection (using Gq DREADD) decreased sucrose intake, while inhibition (using Gi DREADD) increased sucrose intake ($F_{2,31}$=18.891; P=.001). No significant changes in sucrose consumption were observed in the control group without DREADD expression (P=.50).

Conclusions: This study shows that projections from the VP to the AcbSh modulate sucrose intake but do not affect the motivation to work for sucrose. Chemogenetic activation reduced sucrose consumption, while inhibition increased it, suggesting that distinct neural circuits within the VP-AcbSh pathway may differentially regulate feeding behaviors. These findings highlight the role of this pathway in the consumption of palatable foods and indicate that future research should consider factors such as sex, food macronutrient composition, and specific neural subpopulations to better understand their role in feeding behavior.

(JMIRx Bio 2025;3:e68519) doi:10.2196/68519

KEYWORDS

ventral pallidum; nucleus accumbens shell; chemogenetics; sucrose; feeding behavior; food motivation; palatable food; DREADD; designer receptors exclusively activated by designer drugs

Introduction

The neural control of food intake and energy balance involves interactions between homeostatic and nonhomeostatic systems. Traditionally, homeostatic regulation was attributed to hypothalamic and brainstem circuits responding to metabolic signals [1].

Critically, ventral striatopallidal structures, including the nucleus accumbens shell (AcbSh) and ventral pallidum (VP), exert a major influence on ingestive behavior by acting on some of these structures, mainly the lateral hypothalamus (LH). Inhibition of AcbSh neurons through gamma-aminobutyric acid (GABA) agonists or glutamate antagonists elicits intense feeding responses and activates LH neurons, as evidenced by increased *Fos* expression [2]. The AcbSh projects to both the LH and VP, with unilateral lesions of either structure attenuating AcbSh-induced feeding [3]. The LH also modulates AcbSh activity directly through neurotransmitters like orexin and melanin-concentrating hormone, and indirectly via subcortical relay regions such as the VP [4,5]. Relatedly, blockage of GABA receptors in the VP elicits food intake in satiated rats [2], and this feeding presents a clear fat preference [6].

Recent studies have suggested a role of sex in the mediation of sucrose consumption. In female rats, optogenetic stimulation of AcbSh projections to the VP decreased sucrose intake and altered its hedonic value [7]. Additionally, increased sucrose intake has been reported in male rats, but not female rats, because of chemogenetic activation of GABAergic projection neurons in the VP [8].

Both the AcbSh and VP regulate food intake. Notably, the relationship between the VP and AcbSh is that of a loop, and the role that projections between the 2 play in feeding remains understudied. The directionality of the circuit is relevant, as projections from the AcbSh to the VP have different effects compared to projections from the VP to the AcbSh [9]. Additionally, as mentioned above, sex differences have been reported when modulating the projections of the VP [8]. Here, we aim to study the role that chemogenetic activation or inhibition of projections from the VP to the AcbSh have on the motivation to work for sucrose and on the consumption of sucrose in female rats. We hypothesize that chemogenetic modulation of the VP-AcbSh pathway, either inhibition or excitation, will alter the motivation to work for sucrose and sucrose consumption.

Methods

Subjects

A total of 36 female Sprague-Dawley rats (Envigo) were used for these studies; they were 75 days old and weighed 250-300 g (at the time of arrival). After all the procedures described in this section were completed, the final number of rats per group were as follows: Gi (inhibitory G protein) DREADD (designer receptors exclusively activated by designer drugs), n=11; Gq (excitatory G protein) DREADD, n=10; and no DREADD, n=12. All rats were pair-housed in temperature- and humidity-controlled rooms with a 12:12 light-dark cycle. In their home cages, rat pairs had access to chewing bones and a polyvinyl chloride pipe hut. After arrival at the facility, the rats were allowed to acclimate to the colony room for at least 1 week before starting behavioral testing; during this time, the rats were handled once a day by researchers. The rats were also handled regularly for the duration of the behavioral experiments. All rats had ad libitum access to food and water for the duration of the experiments. Behavioral testing took place during the light cycle between 10:00 AM and 5:00 PM.

Ethical Considerations

The experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Parkside and were in accordance with the guidelines on animal care and use of the National Institutes of Health.

Surgeries

Surgeries were performed using standard, aseptic, flat-skull stereotaxic techniques under isoflurane anesthesia (5% induction and 2% maintenance) delivered by a precision vaporizer. Once a stable plane of anesthesia was achieved, a sterile eye ointment was applied to both eyes (to prevent corneal desiccation), the analgesic was administered, the scalp was prepped for an incision (hair trimming with alcohol and iodine scrub), an incision was used to expose the skull, and burr holes were created above the target structures for the injection of adeno-associated viruses (AAVs).

An AAV, double-floxed inverse open reading frame (DIO) construct containing an inverted form of either Gi (AAV5 AAV-hSyn-DIO-hM4D(Gi)-mCherry; Addgene) or Gq (AAV5 AAV-hSyn-DIO-hM3D(Gq)-mCherry; Addgene) DREADD was injected into the VP (from bregma: anterior posterior: –0.2 mm; medial lateral: ±1.8 mm; and dorsal ventral: –8.7 mm). A retrograde AAV-Cre viral vector (AAVrg pENN.AAV.hSyn.HI.eGFP-Cre.WPRE.SV40; Addgene) was injected into the AcbSh (from bregma: anterior posterior: 1.6 mm; medial lateral: ±0.8 mm; and dorsal ventral: –8.1 mm). Injections were performed using a Harvard micropump, Hamilton microsyringes connected to fluid-filled flexible tubing, and Plastics One injectors for a final volume of 1 uL at an injection rate of 300 nL per minute.

For pain management, meloxicam (2 mg/kg, subcutaneous) was administered during the surgery and 24 hours later. Triple antibiotic was applied around the incision after closure using wound clips. Clips were removed 7 to 10 days after the surgery.

The rats were allowed to recover for 2 weeks before behavioral testing.

Clozapine-N-Oxide Preparation

Clozapine-N-oxide (CNO) was obtained from the National Institute on Drug Abuse Drug Supply Program. CNO was administered intraperitoneally 20 minutes before behavioral testing at a dose of 3.0 mg/kg. CNO was freshly prepared daily by dissolving it in 100% dimethyl sulfoxide (DMSO) and then diluting it with sterile water to a final concentration of 6% DMSO. A 6% DMSO solution in sterile water was used as the vehicle control.

Sucrose Access Under a Progressive Ratio Operant Task

The rats were trained in a progressive ratio (PR) operant task using identical, standard, twin-lever operant chambers (Med-Associates) housed within sound-attenuating chambers. First, the animals got 2 daily, 30-minute, magazine training sessions in the operant boxes, during which reinforcers (45-mg, sucrose, banana-flavored Dustless Precision Pellets; BioServe) were presented at 1-minute intervals, with a “click” generated at the same time as food delivery. Next, the rats were shaped to press the lever and then placed on a fixed ratio (FR) 1 reinforcement schedule for 2 days. The rats got one session of training on an FR2 schedule, followed the next day by one on an FR4 schedule. The rats were then switched to a PR6 schedule, which continued for the remainder of the experiment. Each day, the rats were placed into operant chambers with the house light on and both levers extended; only one lever was associated with the sucrose reward, although presses on both levers were recorded. The first response on the correct lever was followed by a sucrose pellet reward, paired with the operation of the clicker. The number of responses required to earn each subsequent sucrose pellet was increased by 6 after each reinforcer, so that 7 responses were required to earn the second pellet, 13 to earn the third, and so on. The time of each lever press was recorded. Each session continued until a 3-minute pause in responding occurred—a cutoff value that has been used in other studies [10,11]—or 60 minutes had elapsed, at which time the house lights were turned off, the levers were retracted, and the rats were removed from the chambers. The animals ran for 5 days on the PR6 schedule prior to drug treatment. After that, and 20 minutes before behavioral testing, the rats were injected with either CNO (3.0 mg/kg) or the vehicle. All rats were administered 2 injections of CNO on 2 different days and 2 injections of the vehicle, also on 2 different days.

Free-Access Sucrose Consumption Test

The rats were placed in individual home cages with wired bottoms and given access to a 20% sucrose solution for 60 minutes. This procedure was repeated over 2 consecutive days to acclimate the rats to the sucrose solution and minimize neophobia. After these 2 days, the rats were administered with either CNO (3.0 mg/kg) or the vehicle 20 minutes before being placed in the individual home cages. The sucrose bottles were weighed before and after the experiment to measure consumption. As described before, all rats got 2 CNO and 2 vehicle injections, with each injection on a different day.

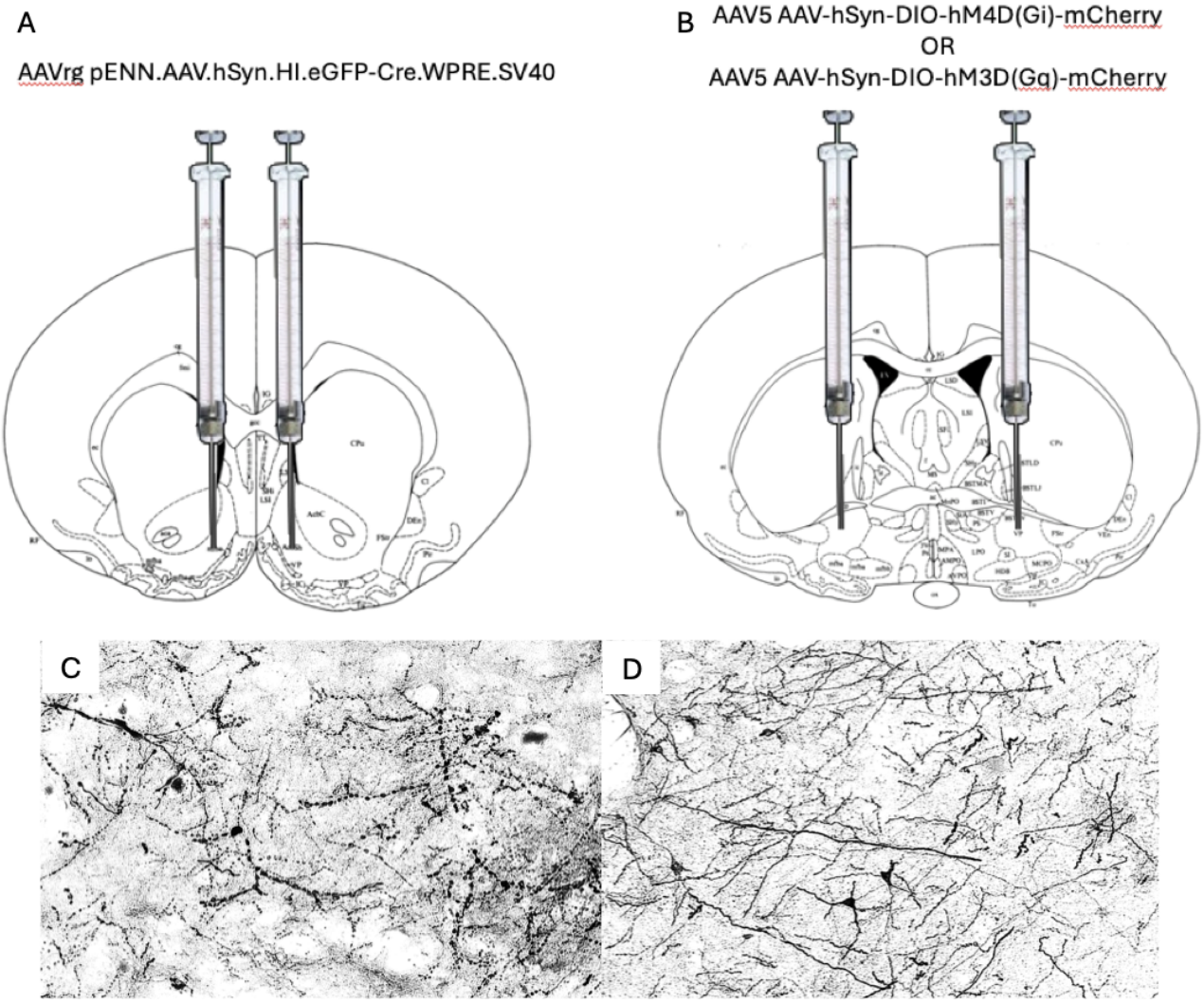
Perfusion and Tissue Processing

After completing the behavioral experiments, the rats were anesthetized with 5% isoflurane and transcardially perfused with 0.9% saline followed by 4% formaldehyde (pH=7.4) for fixation. The brains were extracted, postfixed in 4% formaldehyde for 24 hours at 4°C, and immersed in increasing concentrations of sucrose solutions every 24 hours (10%, 20%, and then 30% sucrose in 0.1 M phosphate-buffered saline [PBS], pH=7.4) at 4°C over the course of 3 days. The brains were then encased in Tissue-Plus O.C.T. (Fisher HealthCare), frozen using dry ice, and subsequently sectioned in the coronal plane (45 µm) using a cryostat.

Immunohistochemistry

The accuracy of DREADD expression in the VP and AcbSh was assessed using immunohistochemistry aimed at visualizing mCherry protein in DREADD-expressing neurons using procedures described previously [12]. Free-floating coronal sections from the VP and AcbSh were first rinsed 3 times in 0.1 M PBS (pH=7.4). Endogenous peroxidase activity was blocked by incubating sections in 1% H_2O_2 for 10 minutes, followed by 3 additional rinses. To prevent nonspecific binding of the secondary antibody, sections were incubated in 0.1 M PBS containing 0.4% Triton X-100 (TX) and 2.5% normal donkey serum (NDS; Jackson ImmunoResearch Laboratories, Inc). Sections were then incubated overnight at room temperature with the primary antibody (rabbit anti-mCherry; Abcam; diluted 1:30,000) in 0.1 M PBS + 0.4% TX + 1% NDS. Then, sections were rinsed again before being incubated for 1 hour in a biotinylated, donkey, anti-rabbit secondary antibody (Jackson ImmunoResearch Laboratories, Inc; diluted 1:500) in 0.1 M PBS + 0.4% TX + 1% NDS. Peroxidase staining was obtained by using a standard avidin-biotin procedure using the Vectastain Elite ABC Kit (Vector Laboratories, Inc; diluted 1:1000 for A and B). Chromogenic reaction occurred by incubating sections in a 0.1 M PBS solution containing 0.02% 3,3’-diaminobenzidine tetrahydrochloride and 0.012% H_2O_2. Sections were rinsed and stored at 4°C until mounted, air dried, and covered with slips using a toluene-based mounting medium (Permount; Thermo-Fisher Scientific). Bright-field images containing the VP or AcbSh were captured using a Zeiss Axioscan light microscope and were analyzed by an experimenter blinded to the experimental groups. The location of mCherry expression was confirmed using a rat brain atlas [13]. A schematic representation of the approach and representative mCherry pictures can be found in Figure 1 [14].

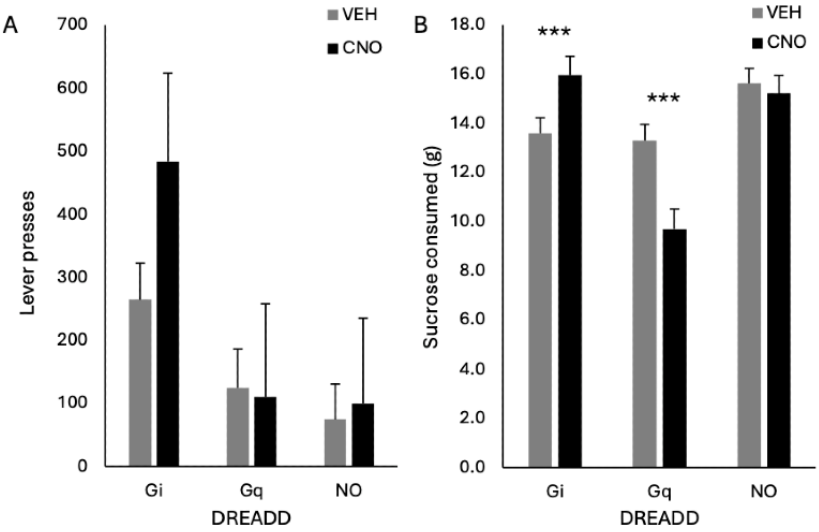
Figure 1. (A) A retrograde AAV-Cre viral vector was injected into the AcbSh. (B) An AAV DIO construct containing an inverted form of either Gi or Gq DREADD was injected into the VP (adapted from Paxinos and Watson [14]). Representative AcbSh (C) or VP (D) 10× microphotograph of mCherry immunohistochemistry. AAV: adeno-associated virus; AcbSh: nucleus accumbens shell; DIO: double-floxed inverse open reading frame; DREADD: designer receptors exclusively activated by designer drugs; Gi: inhibitory G protein; Gq: excitatory G protein; VP: ventral pallidum.



Results

A 2-way ANOVA was performed to evaluate the effects of DREADD type (Gq, Gi, or no DREADD) and drug administered (vehicle or CNO) on lever presses in a sucrose PR task. The results indicated no significant main effect for DREADD type ($F_{2,31}$=2.421; P=.10); no significant main effect for drug administered ($F_{1,31}$=2.004; P=.17); and no significant interaction between DREADD type and drug administered ($F_{2,31}$=1.780; P=.18; Figure 2A).

Figure 2. (A) CNO administration did not affect motivation to work for sucrose, as measured using a progressive ratio task in non–food-deprived, DREADD-expressing rats (Gi and Gq) and control rats (no DREADD). (B) Non–food-deprived rats expressing inhibitory (Gi), excitatory (Gq), or no DREADD were given 1 hour to consume a 20% sucrose solution after being injected with either the vehicle or CNO. CNO-induced chemogenetic inhibition of the VP-AcbSh pathway increased sucrose consumption in rats (*P*=.001), excitation decreased it (*P*=.001) and had no effect on rats not expressing DREADD (*P*=.50). CNO: clozapine-N-oxide; DREADD: designer receptors exclusively activated by designer drugs; Gi: inhibitory G protein; Gq: excitatory G protein; VEH: vehicle.



A 2-way ANOVA was performed to evaluate the effects of DREADD type (Gq, Gi, or no DREADD) and drug administered (vehicle or CNO) on 20% sucrose consumption in non–food-deprived rats. The results indicated a significant main effect for DREADD type ($F_{2,31}$=11.170; *P*=.001); no significant main effect for drug administered ($F_{1,31}$=3.148; *P*=.09); and a significant interaction between DREADD type and drug administered ($F_{2,31}$=18.891; *P*=.001; Figure 2B).

Post hoc testing using Bonferroni correction for multiple comparisons indicated that sucrose consumption was significantly higher for rats expressing Gi DREADD when CNO was administered than when the vehicle was administered (*P*=.003). Additionally, sucrose consumption was significantly lower for rats expressing Gq DREADD when CNO was administered than when the vehicle was administered (*P*=.001). There was no significant difference between the sucrose consumption of rats expressing no DREADD administered with either CNO or the vehicle (*P*=.50; Figure 2B).

Discussion

In female rats, chemogenetic excitation or inhibition of projections from the VP to the AcbSh influenced consumption of a 20% sucrose solution but had no effect on the motivation to work for a sucrose pellet, as measured using a PR task. Specifically, chemogenetic activation of projections from the VP to the AcbSh in non–food-deprived female rats decreased consumption of the 20% sucrose solution. Conversely, chemogenetic inhibition of the same projection increased consumption of the 20% sucrose solution.

In contrast, Scott et al [8] reported that chemogenetic activation of VP projection neurons resulted in no significant changes in rat chow or sucrose consumption. This apparent discrepancy between the 2 studies can be explained by multiple reasons. Possibly the most crucial difference between the 2 studies is that, here, we used a dual vector approach to express DREADD in VP neurons that project to the AcbSh, while Scott et al [8] used a single vector approach, leading to all GABAergic VP projection neurons expressing DREADD. Thus, here, chemogenetic manipulations affected a small subset of VP projection neurons, namely those that project to the AcbSh, while in the study conducted by Scott et al [8], all VP projections were affected by chemogenetic modulation. It is nonetheless informative that we observed different behavioral effects, as this suggests that different VP efferents might have a variety of behavioral effects. This matter could be addressed by future studies dissecting the role of each VP efferent. Additional studies should also consider the sex differences noted by Scott et al [8].

Other differences to consider between the 2 studies include the concentration of sucrose used in the free-access test, as we used a 20% concentration while Scott et al [8] used 10%; the fact that our rats remained pair housed as opposed to single housed; and the differences in rat strain, as they used Long-Evans rats and we used Sprague-Dawley rats. Additionally, there were also differences in the DREADD agonist used: JHU37160 versus CNO in our experiment. While all these differences possibly contributed to some extent to the different behavioral results between the 2 studies, we consider that the most likely difference stems from the targeting of all GABAergic VP projecting neurons in Scott et al [8] versus only VP neurons projecting to the AcbSh in this study.

The directionality of the VP-AcbSh pathway has also been studied by Smedley et al [9]. Interestingly, this group saw no effect on free feeding on male rats when the projections from the VP to the AcbSh were chemogenetically inhibited. Besides the sex differences in the subjects, it is also notable that Smedley et al [9] measured the intake of standard rat chow. In contrast, here, we measured the consumption of a 20% sucrose solution. It is then possible that either or both factors, sex and food stuff,

might contribute to the different behavioral results observed. Thus, it appears that projections from the VP to the AcbSh mediate sucrose consumption but not motivation to work for sucrose. Future studies looking at other VP effects might be able to dissect which projections are involved in the motivation to work for sucrose and other palatable foods.

Additionally, it has been reported that pharmacological activation of the VP leads to increased preference for fat consumption [6]. In contrast, the food used in this study contained mainly carbohydrates, 94% in the case of the sucrose pellets used in the PR task and 20% in the case of the free-access task. It is then plausible that identical manipulations of the VP-AcbSh pathway could result in different behavioral effects if fats instead of carbohydrates were used as food rewards. Future studies should consider the possibility that different behavioral effects might be observed by using fats or offering a choice of different macronutrients.

Further, it has been described that arkypallidal neurons located in the VP inhibit AcbSh neurons and increase consumption of a 5% sucrose reward in mice [15]. In contrast, in this study, activation of the VP-AcbSh pathway led to a decrease in the consumption of the 20% sucrose reward in rats. This discrepancy could be caused, at least in part, by the difference in the nature of the projection neurons recruited and their putative roles, as we targeted all VP neurons projecting to the AcbSh, while Vachez et al [15] specifically targeted ventral arkypallidal neurons. It is then possible that the behavioral effects of modulating the whole VP-AcbSh pathway, as done here, differ from that of specific neural subpopulations. Also intriguing is the possibility that the VP-AcbSh pathway underlies different behavioral outcomes depending on the timing of the stimulation applied. Vachez et al [15] used phasic optogenetic stimulation, while we used more tonic chemogenetic manipulations. Future studies should contemplate the examination of phasic versus tonic stimulation in this pathway.

In conclusion, our findings indicate that the VP-AcbSh pathway mediates the consumption of a palatable sucrose solution. Chemogenetic manipulation of VP projections to the AcbSh selectively influenced sucrose intake without affecting motivation to work for sucrose pellets, suggesting that distinct VP efferents play differential roles in feeding behavior versus food-seeking motivation. Additionally, the findings indicate a nuanced role for the VP-AcbSh pathway in modulating the intake of specific macronutrients. Future studies that dissect the role of the VP-AcbSh pathway should consider variables such as macronutrient profile, sex, and neural subpopulations as well as their possible interactions.

Acknowledgments

The authors would like to thank the College of Natural and Health Sciences for the support; the National Institute on Drug Abuse Drug Supply Program for providing the clozapine-N-oxide; and the following students for their help with this work: Megane Beaupre, Ari Hjelmeseth, Miranda Johnson, Hedi Morris, Zach Wilson, Aryan Patel, Mark Huckeby, and Dylan Jensen.

Conflicts of Interest

None declared.

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Abbreviations

AAV: adeno-associated virus
AcbSh: nucleus accumbens shell
CNO: clozapine-N-oxide
DIO: double-floxed inverse open reading frame
DMSO: dimethyl sulfoxide
DREADD: designer receptors exclusively activated by designer drugs
FR: fixed ratio
GABA: gamma-aminobutyric acid
Gi: inhibitory G protein
Gq: excitatory G protein
LH: lateral hypothalamus
NDS: normal donkey serum
PBS: phosphate-buffered saline
PR: progressive ratio
TX: Triton X-100
VP: ventral pallidum

Edited by O Singh; submitted 07.11.24; peer-reviewed by D Wirtshafter, J Grimm; comments to author 01.01.25; revised version received 08.01.25; accepted 18.01.25; published 08.03.25.

Please cite as:
Peroutka M, Rivero Covelo I
Effects of Ventral Pallidum–Nucleus Accumbens Shell Neural Pathway Modulation on Sucrose Consumption and Motivation in Female Rats: Chemogenetic Manipulation
JMIRx Bio 2025;3:e68519
URL: https://bio.jmirx.org/2025/1/e68519
doi:10.2196/68519
PMID:

Original Paper

Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran: Cross-Sectional Study

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Abstract

Background: Ticks are well-known ectoparasites of domestic animals, causing significant economic losses and playing a crucial role in the transmission of pathogens within the livestock industry worldwide, including in Iran. Understanding the distribution and diversity of ticks is essential for effective control strategies, especially in regions like Tehran province, where livestock plays a vital role in the economy.

Objective: This study aimed to determine the frequency and distribution of livestock ticks across different seasons and climatic zones in Tehran province.

Methods: In 2019, a total of 1623 domestic animals infested with ticks were examined, including chickens, sheep, camels, cows, pigeons, and dogs. A total of 806 ticks were collected, comprising 121 (15%) soft ticks and 685 (85%) hard ticks. Tick species were identified and categorized based on their occurrence in mountainous and plain climate regions.

Results: Out of the 806 collected ticks, 44.8% (n=361) were found in the mountainous region and 55.2% (n=445) were found in the plain region. The most abundant species was *Rhipicephalus sanguineus* (n=307, 38.1%), while *Rhipicephalus (Boophilus) annulatus* was the least common (n=3, 0.4%). Seasonal variation indicated peak infestation in the spring (n=486, 60.3%) and the lowest infestation in the winter (n=77, 9.6%).

Conclusions: The study highlights the significant diversity and abundance of both soft and hard ticks in livestock across various regions of Tehran province. These findings emphasize the need for targeted tick control measures, considering the differences in tick distribution between mountainous and plain climate regions.

(JMIRx Bio 2025;3:e69542) doi:10.2196/69542

KEYWORDS

impact of climate; seasonal change; frequency; livestock; ticks; Tehran

Introduction

Ticks are of outstanding medical and veterinary importance because they transmit severe and dangerous diseases to humans and animals [1]. In humans, most diseases caused by spirochetes and rickettsia are transmitted by ticks. In addition, these arthropods cause severe diseases such as paralysis, encephalitis, and tularemia [2]. Moreover, ticks cause livestock financial losses worldwide and in Iran, where the annual impairment caused by ticks is estimated at US $13.9 to US $18.7 billion [3].

Ticks on livestock cause localized bite-site lesions and systemic effects. They can lead to anemia, paralysis, and even death by transmitting diseases like theileriosis and babesiosis [4]. On the other hand, global climate change has significantly impacted the stability and distribution of their life cycles [5], and climate conditions are the most critical factor determining tick distribution [6]. The tick-borne diseases associated with wildlife and climate change favor the re-emergence of diseases and the possible risk of the emergence of new ones [7-9].

Although the parasitic fauna of *Argasidae* and *Ixodidae* ticks and some of their ecological characteristics have been studied in a few areas of Iran [10], the various species from all regions of Iran in general and Tehran province in particular and especially the seasonal activity are not thoroughly studied. Tehran province is located between mountainous and plain regions [11]. Three factors—humid westerly winds, the province's extent, and the Alborz mountain range—play an essential role in Tehran province's climate. The Alborz mountain range has tempered the climate of Tehran province. It is mountainous: temperate in the north and semiarid in the lowlands. Tehran province features a diverse geography, encompassing mountainous and plain areas with distinct climates. In the mountainous region, such as Shemiranat, the average annual temperature ranges from 10 °C to 12 °C, with increased precipitation of 350 to 400 mm annually. Winters are cold, with substantial snowfall, while summers are mild. Conversely, the plain areas like Varamin have hotter conditions, averaging from 18 °C to 20 °C annually, with lower rainfall of 150 to 250 mm. These plains experience hot summers and milder winters, characterized by a semiarid climate [12,13].

This study was conducted to determine the seasonal and climatic frequency of tick species in Tehran province on the body surface of livestock, involving chickens, camels, cattle, dogs, pigeons, and sheep in different areas. The importance and current status of tick control in this region will become more evident when we know the distribution of ticks, their location, and presence in each region, and the epidemiological situation can be determined. In Tehran province, so far, not much research has been done on the climatic and seasonal distribution of different species of ticks.

Also, the published information about livestock infested with ticks in this area is not complete, so the purpose of this study is to provide an accurate scientific report of the situation of livestock infested with hard and soft ticks in different climates and during different seasons of the year; this information can be used in macroplanning to combat foreign parasites.

Methods

Geographical Area

The study was conducted in two different environments: plain and mountainous regions within 20 selected villages in Tehran province, which were located between 34° to 36.5° N and 50° to 53° E.

Sampling

The sample size was calculated using the Cochran formula for prevalence studies. Given an estimated prevalence (p) of 30% and a precision (d) of 4.5%, the final sample size was determined to be 800 ticks, ensuring statistical reliability [14]. To ensure representative sampling, a cross-sectional study was conducted, covering both mountainous and plain regions. The selection of livestock was randomized among those showing visible tick infestation, with veterinary supervision ensuring consistency in sample collection across different geographical zones. The chosen method aligns with established epidemiological studies on tick distribution.

The prevalence value (p=0.3) was selected based on prior studies on tick prevalence in similar regions in Iran, indicating an estimated infestation rate of 30%. The margin of error (d=0.045) was determined considering a 95% confidence level, ensuring a balance between precision and the feasibility of sample collection.

Study Area

The study was performed in two separate climatic zones, including 6 mountainous villages and 14 plain villages. After collecting geographical and ecological information, 1623 domestic animals were selected [14]. Using a cross-sectional study design, the distribution of ticks was studied in different study areas from spring to the end of winter of 2019. Ticks were collected using curved forceps from the host body (livestock's earlobes, groin, tail base, and back, and poultry's underarms, groin, and abdomen). To identify the collected ticks' genus and species, valid diagnostic keys were used [15]. The tick species were identified using the diagnostic keys outlined by Jongejan et al [15] and Camicas et al [16], which provide detailed morphological descriptions and illustrations for the identification of both soft and hard ticks. These keys are widely recognized for their accuracy and reliability in the identification of tick species in the Middle East and neighboring regions [16,17]. Based on the distribution of ticks in the study area, the prepared maps, the identification of infested carriers, climate conditions, and host animals of these tick species were analyzed, providing an understanding of the existing situation in the province [16-20]. Two professional stereo microscopes were used to

identify tick species: the Leica S9i and the Zeiss Stemi 508. The Leica S9i has 10× to 60× magnification and includes a 10 megapixel camera, while the Zeiss Stemi 508 offers 8× to 50× magnification with superior optical quality. Both tools are essential for the detailed investigation of tick morphology in entomological research.

Results

Determining the Frequency of Livestock Ticks by Climate Type

In this study, 1623 domestic animals infested with ticks were studied, including chickens, camels, cattle, dogs, pigeons, and sheep. A total of 806 ticks were collected, and 685 (85%) and 121 (15%) of them were detected as hard ticks and soft ticks, respectively. The distribution of collected ticks indicates that out of 806 collected ticks, 361 (44.8%) belonged to the mountainous region, and 445 (55.2%) belonged to the plain region (Table 1). In the mountainous region, the genus *Rhipicephalus*, with 51.2% (185/361), was the most frequent, and the genera *Hyalomma* and *Ornithodoros* were the least frequent. In the plain region, the genus *Hyalomma*, with 66.3% (295/445), was the most frequent, and the genera *Haemaphysalis* and *Boophilus* were the least frequent (Table 1).

Table 1. The number and frequency of ticks caught by genera according to climatic topography, Tehran province, 2019.

Genera	Mountainous region, n (%)	Plain region, n (%)	Total, n (%)
Rhipicephalus (n=307)	185 (60.3)	122 (39.7)	307 (100)
Hyalomma (n=295)	0 (0)	295 (100)	295 (100)
Argas (n=102)	93 (91.2)	9 (8.8)	102 (100)
Haemaphysalis (n=80)	80 (100)	0 (0)	80 (100)
Ornithodoros (n=19)	0 (0)	19 (100)	19 (100)
Rhipicephalus (Boophilus) (n=3)	3 (100)	0 (0)	3 (100)
Total (n=806)	361 (44.8)	445 (55.2)	806 (100)

Argas persicus from the genus *Argas*, *Rhipicephalus bursa* and *Rhipicephalus sanguineus* from the genus *Rhipicephalus*, and all species from the genera *Boophilus* and *Haemaphysalis* were found in the mountainous region of Tehran province. Among the ticks found in the mountainous region, *R sanguineus*, with 48.8% (176/361), was the most frequency. In comparison, *Rhipicephalus (Boophilus) annulatus*, with 0.8% (3/361), was the least frequent (*Hyalomma marginatum*, *Hyalomma asiaticum*, *Hyalomma dromedarii*, *Hyalomma anatolicum*, *Ornithodoros lahorensis*, *Argas reflexus*, and *Hyalomma detritum* were not found in the mountainous region). All *Ornithodoros* species, all *Hyalomma* species, *A reflexus*, and *R sanguineus* were collected from the plain region. Among the tick species found in the plain region, *Hy marginatum*, with 34.3% (152/445), was the most frequent, and *Hy detritum*, with 1.3% (6/445), was the least frequent (*A persicus*, *Haemaphysalis sulcata*, *Haemaphysalis inermis*, *Haemaphysalis erinacei*, *R bursa*, and *R (B) annulatus* were not found in the plain region; Table 2).

The results show that 38.7% (628/1623) and 6.13% (995/1623) of studied animals belonged to the mountainous and plain regions, respectively (Table 3). Of the 230 infested animals, 97 (42.2%) and 133 (57.8%) belonged to the mountainous and plain regions, respectively.

Table 2. The number and frequency of ticks caught by species according to climatic topography, Tehran province, 2019.

Species	Mountainous region, n (%)	Plain region, n (%)	Total, n (%)
Rhipicephalus sanguineus (n=298)	176 (59.1)	122 (40.9)	298 (100)
Hyalomma marginatum (n=152)	0 (0)	152 (100)	152 (100)
Argas persicus (n=93)	93 (100)	0 (0)	93 (100)
Hyalomma anatolicum (n=21)	0 (0)	21 (100)	21 (100)
Hyalomma dromedarii (n=49)	0 (0)	49 (100)	49 (100)
Hyalomma asiaticum (n=67)	0 (0)	67 (100)	67 (100)
Haemaphysalis sulcata (n=47)	47 (100)	0 (0)	47 (100)
Ornithodoros lahorensis (n=19)	0 (0)	19 (100)	19 (100)
Haemaphysalis inermis (n=24)	24 (100)	0 (0)	24 (100)
Haemaphysalis erinacei (n=9)	9 (100)	0 (0)	9 (100)
Argas reflexus (n=9)	0 (0)	9 (100)	9 (100)
Rhipicephalus bursa (n=9)	9 (100)	0 (0)	9 (100)
Rhipicephalus (Boophilus) annulatus (n=3)	3 (100)	0 (0)	3 (100)
Hyalomma detritum (n=6)	0 (0)	6 (100)	6 (100)
Total (n=806)	361 (44.8)	445 (55.2)	806 (100)

Table 3. The number and frequency of animals infested by ticks in different climate regions, Tehran province, 2019.

Climate region	Collected ticks (n=806), n (%)	Infested animals (n=230), n (%)	Studied animals (n=1623), n (%)
Mountainous	361 (44.8)	97 (42.2)	628 (38.7)
Plain	445 (55.2)	133 (57.8)	995 (61.3)

When analyzing the data, it was found that domestic animals had the highest rate of tick infestation in the spring, while the lowest rate of infestation was observed in the winter. It indicates a seasonal variation in tick distribution and infestation rates among domestic animals. In the spring, all genera (except *Boophilus*) were found. The genera *Rhipicephalus* and *Hyalomma* were distributed in the summer. In the autumn, the genus *Hyalomma* was the most abundant, and in the winter, the species *A persicus* (belonging to the family of soft ticks) had a high abundance (Table 4). The frequency of all 6 ticks (4 hard ticks and 2 soft ticks) in different seasons of the year is described below.

Table 4. The number and frequency of ticks caught according to the season (n=806), Tehran province, 2019

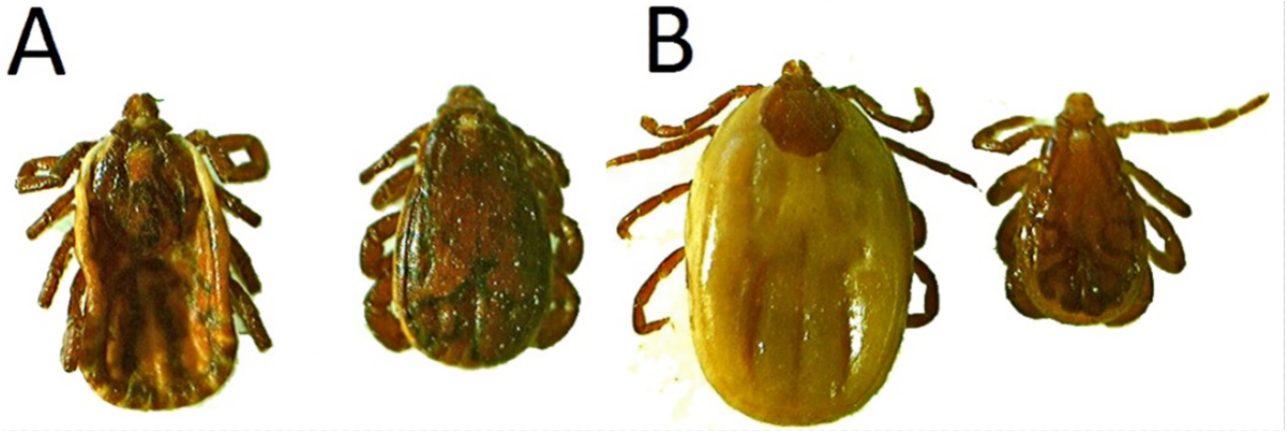
Species	Seasons				
	Spring, n	Summer, n	Autumn, n	Winter, n	Total, n (%)
Rhipicephalus sanguineus	251 (31.1)	23 (2.9)	18 (2.2)	6 (0.7)	298 (37)
Hyalomma marginatum	74 (9.2)	53 (6.6)	14 (1.7)	11 (1.4)	152 (18.8)
Argas persicus	34 (4.2)	0 (0)	41 (5.1)	18 (2.2)	93 (11.5)
Hyalomma asiaticum	33 (4.1)	22 (2.7)	9 (1.1)	3 (0.4)	67 (8.3)
Hyalomma dromedarii	11 (1.4)	13 (1.6)	7 (0.9)	18 (2.2)	49 (6.1)
Haemaphysalis sulcata	28 (3.5)	15 (1.9)	0 (0)	4 (0.5)	47 (5.8)
Hyalomma anatolicum	14 (1.7)	0 (0)	0 (0)	7 (0.9)	21 (2.6)
Ornithodoros lahorensis	12 (1.5)	3 (0.4)	4 (0.5)	0 (0)	19 (2.4)
Haemaphysalis erinacei	9 (1.1)	0 (0)	0 (0)	0 (0)	9 (1.1)
Haemaphysalis inermis	12 (1.5)	0 (0)	12 (1.5)	0 (0)	24 (3)
Argas reflexus	3 (0.4)	0 (0)	6 (0.7)	0 (0)	9 (1.1)
Rhipicephalus bursa	3 (0.4)	0 (0)	0 (0)	6 (0.7)	9 (1.1)
Rhipicephalus (Boophilus) annulatus	0 (0)	3 (0.4)	0 (0)	0 (0)	3 (0.4)
Hyalomma detritum	2 (0.2)	0 (0)	0 (0)	4 (0.5)	6 (0.7)
Genera	13 (N/A[a])	7 (N/A)	8 (N/A)	9 (N/A)	14 (N/A)
Total	486 (60.3)	132 (16.4)	111 (13.8)	77 (9.6)	806 (100)

[a]N/A: not applicable.

Seasonal Activity and Fauna of Rhipicephalus (Hard Ticks)

In this study, 307 ticks of the genus *Rhipicephalus* were caught, which was 38.1% (307/806) of the total sample collected (the highest frequency among the genera). *R sanguineus* of this genus was caught in all seasons, while *R bursa* was only caught in the spring and winter (Figure 1).

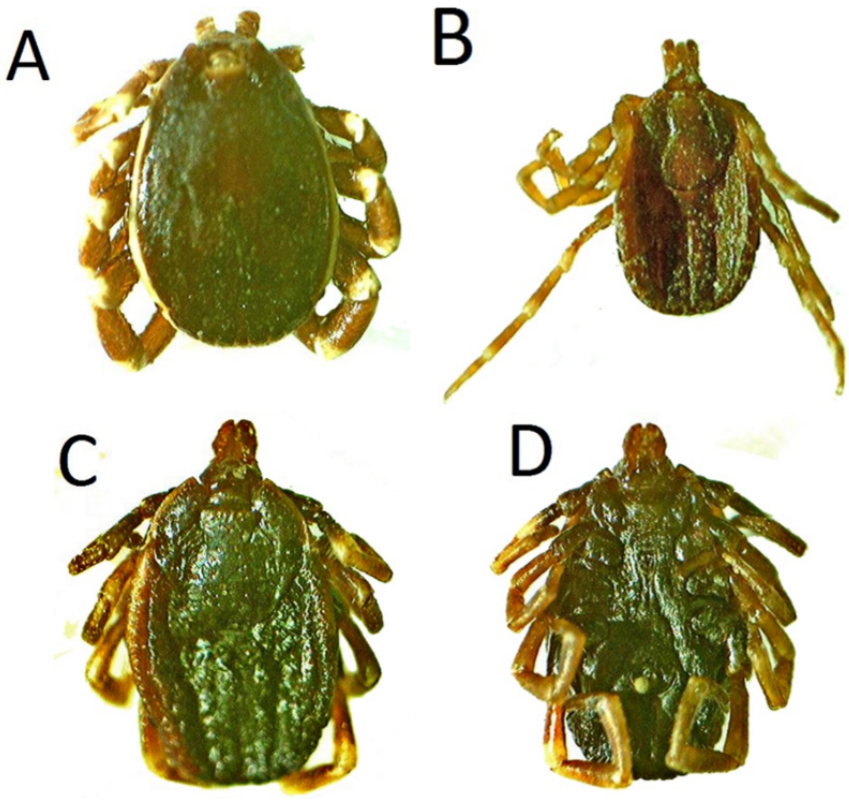
Figure 1. Rear view of (A) *Rhipicephalus sanguineus* (male on the right and female on the left) and (B) *Rhipicephalus bursa* (original; male on the right and female on the left).



Seasonal Activity and Fauna of Hyalomma (Hard Ticks)

The genus *Hyalomma*, with 295 ticks, was the second most abundant genus, accounting for 36.6% (295/806) of the total sample. *Hy marginatum*, *Hy asiaticum*, and *Hy dromedarii* were caught in all seasons, but *Hy anatolicum* and *Hy detritum* were only caught in the spring and winter (Figure 2).

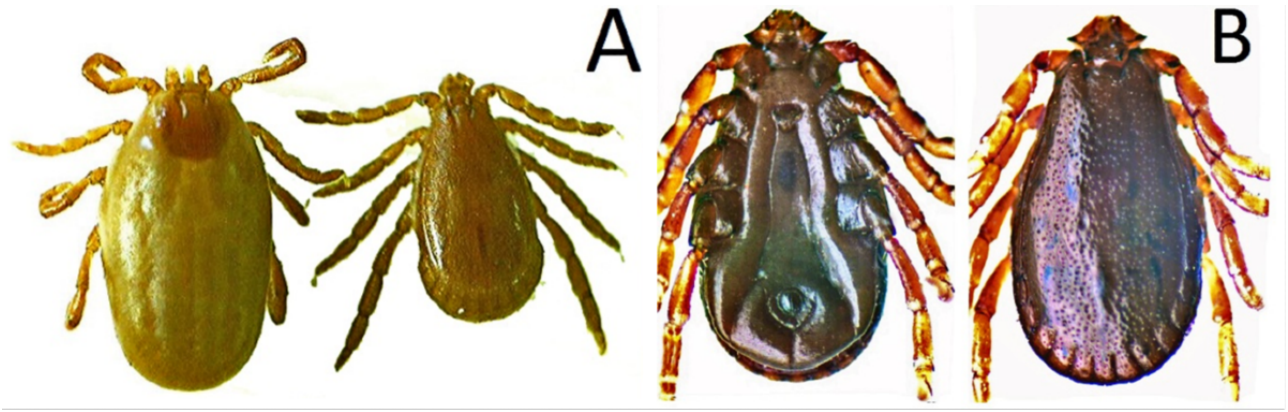
Figure 2. Rear view of (A) male and (B) female *Hyalomma marginatum*; and (C) dorsal view and (D) abdominal view of female *Hyalomma detritum* (original).



Seasonal Activity and Fauna of Haemaphysalis (Hard Ticks)

The genus *Haemaphysalis* was the third genus of the hard tick family detected in this study, and it has 3 species of *Hae sulcata*, *Hae inermis*, and *Hae erinacei*; a total of 80 ticks were caught, with a frequency of 9.9% (80/806). *Hae sulcata* was found in all seasons except for autumn, *Hae inermis* was found in the spring and autumn, and *Hae erinacei* was only caught in the spring (Figure 3).

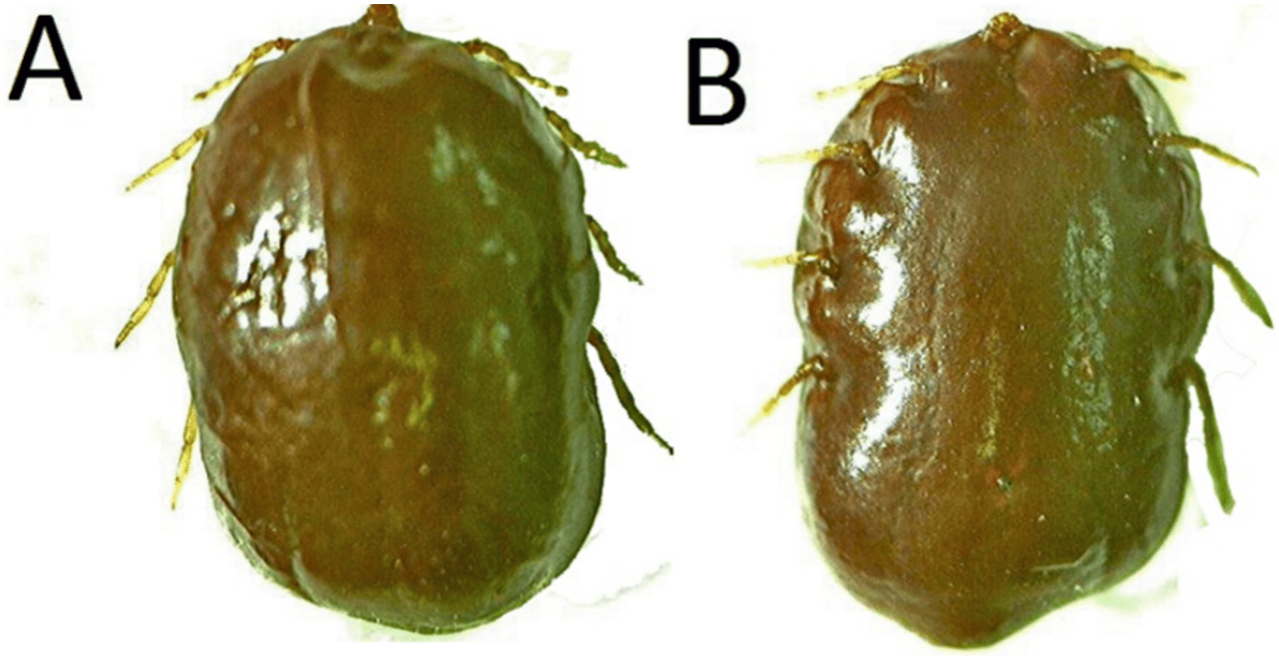
Figure 3. (A) Rear view of *Haemaphysalis sulcata* (male on the right and female on the left) and (B) abdominal view of *Haemaphysalis erinacei* (original; male on the right and female on the left).



Seasonal Activity and Fauna of Rhipicephalus (Boophilus) (Hard Ticks)

In this study, the genera *Boophilus* was only caught in the summer with 1 species, *R (B) annulatus*, with an abundance of 3 ticks (Figure 4).

Figure 4. (A) Rear view and (B) abdominal view of female *Rhipicephalus (Boophilus) annulatus* (engorged; original).

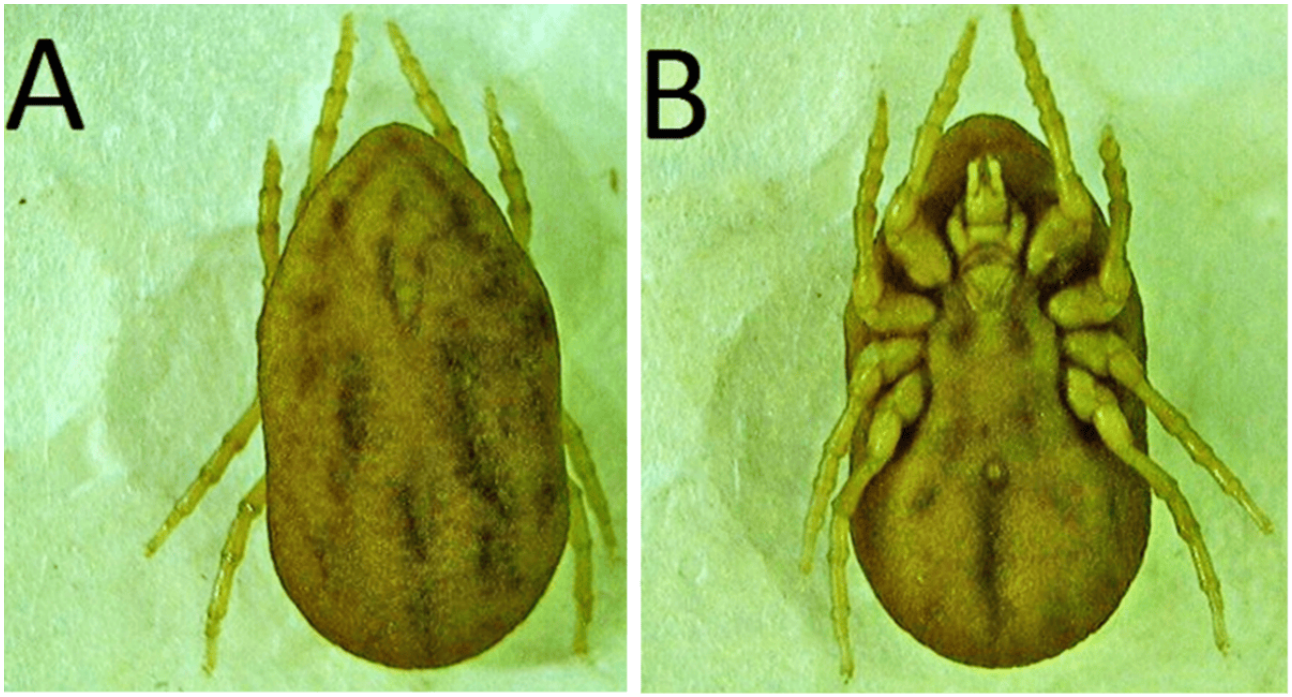


Seasonal Activity and Fauna of Argas and Ornithodoros (Soft Ticks)

In this study, 121 (15% of the total 806) ticks belonging to the soft tick family were caught, which included the genus *Argas* with 2 species, *A persicus* and *A reflexus* (102/121, 84.3%), and the genus *Ornithodoros* with only 1 species, *O lahorensis* (19/121, 15.7%). *A persicus* was caught in all seasons except for the summer, *A reflexus* was caught in the spring and autumn, and *O lahorensis* was caught in all seasons except for the winter (Figures 5 and 6).

Figure 5. (A) Rear view and (B) abdominal view of *Argas persicus* (original).

Figure 6. (A) Rear view and (B) abdominal view of *Ornithodoros lahorensis* (original).



Discussion

Principal Findings

This study is the only codified and comprehensive study on *Ixodidae* and *Argasidae* ticks and their climatic and seasonal activities during 2019 in Tehran province. The *Ixodes* genus was not found in our study because this genus is more distributed in the country's northern provinces, such as Gilan, Mazandaran, and Golestan provinces [21].

A study in Pakistan confirmed the dominance of the tick species of *R (B) annulatus* and *Hy anatolicum*, also prevalent in Tehran province, particularly impacting sheep and cattle. In contrast, a study done in Turkey highlighted that *Ixodes ricinus* is predominantly found in humid regions, which differs from Tehran province's dry climate. Studies from India are also aligned with the findings from Tehran province, showing *R sanguineus* as the most common species. Conversely, Japan reported *Haemaphysalis longicornis* as the most frequent species, influenced by temperate climates. Southern Europe exhibits some similarities with Tehran province, particularly regarding *R sanguineus*; however, it shows higher *I ricinus* prevalence. Northern Europe shows a dominance of *I ricinus* due to temperate climates, differing from Tehran province's dry conditions. In North Africa, tick species like *Hy anatolicum* can be found, which aligns with the findings from Tehran province, while sub-Saharan Africa presents contrasting species like *Amblyomma variegatum*, which thrive in humid climates. Overall, the analysis emphasizes how climate and ecology influence tick species composition and host preferences across regions [22-28].

The observed seasonal trend aligns with the biological cycles of tick species. Hard ticks such as *Rsanguineus* and *Hymarginatum* exhibited peak abundance in the spring and summer due to higher temperatures and increased host activity. Conversely, soft ticks (*Apersicus*) showed resilience during colder months, likely due to their ability to survive in sheltered environments. This seasonal variability highlights the importance of targeted tick control strategies, particularly in warm seasons when the transmission risk of tick-borne diseases is the highest [29-31]. Furthermore, this genus is more distributed in the cold and wet seasons of the year. In our study, different cities in Tehran province did not have high humidity compared to the northern provinces. Therefore, it is assumed that high humidity is a limiting factor in the distribution of this tick in our study area.

In a study in Golestan province, 6 genera and 15 species of ticks, including soft and hard ticks, were reported [32], which is considerably similar to our study conducted in Tehran province. This may be due to the climatic similarities of the two provinces and the proximity and parallelism of research. *A persicus* soft ticks have been caught in Shemiranat city in Tehran province, which has a mountainous climate, with a frequency of 11.5%. *A persicus* is caught in all seasons except for the summer. Its highest frequency was observed in the autumn, which is consistent with studies conducted in the cities of Sanandaj, Boyer-Ahmad, and Bijar in Kurdistan province [33-35].

R sanguineus was the most abundant among the ticks caught in Tehran province. This species has been caught in the cities of Tehran, Islamshahr, Shemiranat, Shahreri, Pakdasht, and Varamin from both plain and mountainous climate regions, which is consistent with other studies carried out in Ghaemshahr, Mazandaran province [36]. In general, the results of this study agree with the studies of other researchers due to the similarity of climate conditions. However, slight differences were observed between the results, which can be attributed to climatic diversity and the susceptibility of different breeds of livestock.

Conclusions

The distribution of collected ticks indicated that out of 806 collected ticks, 44.8% and 55.2% belonged to the mountainous and plain regions, respectively. This study demonstrated significant abundance and diversity of *Ixodidae* and *Argasidae* ticks in livestock in different places of Tehran province.

Acknowledgments

The authors would like to thank the Veterinary Office of Tehran Province and the research vice-chancellor of Shiraz University of Medical Sciences for their cooperation. This study received no grants from commercial, public, or nonprofit entities.

Data Availability

All data obtained from this research are included in the paper's main text.

Authors' Contributions

EA designed the study, collected the ticks, identified tick species, recorded geographic coordinates and area information, wrote the manuscript, and confirmed and submitted the paper.

Conflicts of Interest

None declared.

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Edited by J Ren; submitted 02.12.24; peer-reviewed by J Shen, V Noaman; comments to author 05.02.25; revised version received 11.02.25; accepted 16.02.25; published 31.03.25.

Please cite as:
Abbasi E
Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran: Cross-Sectional Study
JMIRx Bio 2025;3:e69542
URL: https://bio.jmirx.org/2025/1/e69542
doi:10.2196/69542
PMID:

Peer-Review Report

Peer Review of “Discovery of Novel Inhibitors of HMG-CoA Reductase Using Bioactive Compounds Isolated From Cochlospermum Species Through Computational Methods (Preprint)”

Shailee Rasania; Sylvester Sakilay; Sayan Mitra; Randa Salah Gomaa Mahmoud[1]; Julie Moonga[2]

[1]Faculty of Human Medicine, Zagazig University, Zagazig, Egypt

[2]King’s College London, London, United Kingdom

Related Article:

Companion article: https://www.biorxiv.org/content/10.1101/2025.01.19.633828v1

(JMIRx Bio 2025;3:e74084) doi:10.2196/74084

KEYWORDS

statins; phytochemicals; Cochlospermum; hypercholesterolemia; molecular docking

This is a peer-review report submitted for the preprint “Discovery of Novel Inhibitors of HMG-CoA Reductase Using Bioactive Compounds Isolated From Cochlospermum Species Through Computational Methods.”

This review is the result of a virtual, collaborative live review discussion organized and hosted by PREreview and JMIR Publications on February 21, 2025. The discussion was joined by 13 people: 3 facilitators from the PREreview Team, 1 member of the JMIR Publications team, 1 author, and 8 live review participants. The authors of this review have dedicated additional asynchronous time over the course of 2 weeks to help compose this final report using the notes from the live review. We thank all participants who contributed to the discussion and made it possible for us to provide feedback on this preprint.

Summary

Cholesterol is an essential component of cellular membranes and a precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D. However, elevated low-density lipoprotein cholesterol is a major contributor to atherosclerosis and cardiovascular diseases, which are leading causes of morbidity and mortality worldwide. Inhibiting HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase (HMGR) is a key therapeutic strategy for managing hypercholesterolemia, with statins serving as the most widely used competitive inhibitors; however, their prolonged use is associated with adverse effects. This study aims to identify novel, natural inhibitors of HMGR as potential alternatives to statins.

This study [1] used a molecular docking method to investigate the inhibitory potential of 84 phytochemicals from *Cochlospermum planchonii* and *Cochlospermum tinctorium* against human HMGR. Molecular docking is a purely computational technique used to predict how small molecules bind to proteins. Specifically, the author used a semirigid docking approach, meaning that the structure of the receptor was not allowed to change while the phytochemicals and statins were given some degree of flexibility at the binding pocket. The phytochemicals were screened for their drug-likeness and absorption, distribution, metabolism, excretion, and toxicity properties based on Lipinski’s rule of five, and 32 were docked against the enzyme’s HMG-binding site alongside its native ligand and 6 statins as controls. Docking results identified 10 promising inhibitors of HMGR. These compounds, including 3-O-methylellagic acid, all displayed strong binding affinities and interactions that were either comparable to or exceeding those of the statins used as control ligands.

These findings highlight the therapeutic potential of natural compounds in treating hypercholesterolemia. However, as indicated in the manuscript, further in vitro and in vivo experiments will be needed to establish their efficacy and safe therapeutic use.

Concerns and Feedback

All reviewers found that the study was well written and comprehensive. There were no major concerns regarding the techniques or analyses. A few points were made during the discussion and are highlighted below:

- Reviewers appreciated the depth and thoroughness of the search through the literature of peer-review research. Some reviewers were surprised about the date (1991) of some studies related to the high-performance liquid chromatography–UV analysis of phytochemicals identified in the ethanolic and methanolic extract of *C tinctorium* and

wondered whether there may be more recent studies to also consider.
- To increase the reproducibility of the study, some reviewers wondered if it would be possible to make the data and code used to analyze the data openly available.
- The figures and tables are comprehensive and clearly presented, with well-written descriptions. If feasible, reviewers would suggest ways to visually highlight key compounds listed in tables using colors, bold text, or labels. Furthermore, incorporating chemical structures directly within the relevant tables or as supplementary figures would further enhance the understanding of their molecular characteristics and potential interactions.
- While the author acknowledges the need for in vitro and in vivo validation studies, explicitly addressing potential computational limitations—such as docking inaccuracies, semirigid approach versus more flexible ones, or the absence of dynamic modeling—would further strengthen the discussion.
- Some reviewers suggested adapting the part of the study that identified the compounds through literature review into a systematic review.

Concluding Remarks

We thank the author of the preprint for posting their work openly and for allowing the review of their work openly via live review. We also thank all participants of the live review call for their time and for engaging in the lively discussion that generated this review.

Acknowledgments

PREreview and JMIR Publications thank the authors of the preprint for posting their work openly for feedback. We also thank all participants of the live review call for their time and for engaging in the lively discussion that generated this review.

Conflicts of Interest

None declared.

Reference

1. Olatoye TI. Discovery of novel inhibitors of HMG-CoA reductase using bioactive compounds isolated from Cochlospermum species through computational methods. BioRxiv. Preprint posted online on January 22, 2025 2025. [doi: 10.1101/2025.01.19.633828]

Abbreviations

HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A
HMGR: HMG-CoA reductase

Edited by A Schwartz; submitted 17.03.25; this is a non–peer-reviewed article;accepted 17.03.25; published 24.03.25.

Please cite as:
Rasania S, Sakilay S, Mitra S, Mahmoud RSG, Moonga J
Peer Review of "Discovery of Novel Inhibitors of HMG-CoA Reductase Using Bioactive Compounds Isolated From Cochlospermum Species Through Computational Methods (Preprint)"
JMIRx Bio 2025;3:e74084
URL: https://bio.jmirx.org/2025/1/e74084
doi:10.2196/74084
PMID:

Peer-Review Report

Peer Review of “Effects of Ventral Pallidum–Nucleus Accumbens Shell Neural Pathway Modulation on Sucrose Consumption and Motivation in Female Rats: Chemogenetic Manipulation Study”

David Wirtshafter[1], BA, MA, PhD

The University of Illinois at Chicago, Chicago, IL, United States

Related Articles:

Companion article: https://www.biorxiv.org/content/10.1101/2024.11.05.622115v1

Companion article: https://bio.jmirx.org/2025/1/e71629/

Companion article: https://bio.jmirx.org/1/e68519/

(JMIRx Bio 2025;3:e71626) doi:10.2196/71626

KEYWORDS

ventral pallidum; nucleus accumbens shell; chemogenetics; sucrose; feeding behavior; food motivation; palatable food; DREADD; designer receptors exclusively activated by designer drugs

This is a peer-review report submitted for the paper “Effects of Ventral Pallidum–Nucleus Accumbens Shell Neural Pathway Modulation on Sucrose Consumption and Motivation in Female Rats: Chemogenetic Manipulation Study.”

Round 1 Review

General Comments

In this paper [1], the authors present an interesting and well-written paper dealing with the effects of stimulation and inhibition of projections from the ventral pallidum to the nucleus accumbens shell on feeding and food reinforced behaviors. The methods used are cutting edge, and my comments and suggestions are relatively minor.

Specific Comments

Minor Comments

1. In the third paragraph of the Introduction, the sentence beginning with “Parallelly” is very awkward; I am sure there is a way to word this that does not use “parallelly.” Also, the previous sentence could be made clearer as to whether effects on sucrose consumption are found just in female rats.

2. The number of subjects should be listed in the Methods.

3. In the last paragraph of the body of the manuscript, the sentence beginning with “The discrepancies observed across studies of this pathway...” is unfinished, and I am uncertain what the authors intended to say.

4. In discussing the differences between the results observed here and those reported by Vanchez et al [2], is it possible that these may reflect the use of “closed-loop” manipulations linked to the occurrence of licking in the Vanchez et al [2] paper, in contrast to the continuous modulation produced here by the use of the DREADD (designer receptors exclusively activated by designer drugs) technique? Also, in this section, the authors could be a bit clearer as to why the techniques used by Vanchez et al [2] would be expected to label a different subpopulation of cells than was the case in this study.

Conflicts of Interest

None declared.

References

1. Peroutka M, Rivero Covelo I. Effects of ventral pallidum–nucleus accumbens shell neural pathway modulation on sucrose consumption and motivation in female rats: chemogenetic manipulation study. JMIRx Bio 2025;3(1):e68519 [FREE Full text] [doi: 10.2196/68519]

2. Vachez YM, Tooley JR, Abiraman K, Matikainen-Ankney B, Casey E, Earnest T, et al. Ventral arkypallidal neurons inhibit accumbal firing to promote reward consumption. Nat Neurosci 2021 Mar;24(3):379-390 [FREE Full text] [doi: 10.1038/s41593-020-00772-7] [Medline: 33495635]

Abbreviations

DREADD: designer receptors exclusively activated by designer drugs

Edited by O Singh; submitted 22.01.25; this is a non–peer-reviewed article;accepted 22.01.25; published 08.03.25.

Please cite as:
Wirtshafter D
Peer Review of "Effects of Ventral Pallidum–Nucleus Accumbens Shell Neural Pathway Modulation on Sucrose Consumption and Motivation in Female Rats: Chemogenetic Manipulation Study"
JMIRx Bio 2025;3:e71626
URL: https://bio.jmirx.org/2025/1/e71626
doi:10.2196/71626
PMID:

Peer-Review Report

Peer Review of “Effects of Ventral Pallidum–Nucleus Accumbens Shell Neural Pathway Modulation on Sucrose Consumption and Motivation in Female Rats: Chemogenetic Manipulation Study”

Jeffrey Grimm

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Related Articles:

Companion article: https://www.biorxiv.org/content/10.1101/2024.11.05.622115v1

Companion article: https://bio.jmirx.org/2025/1/e71629/

Companion article: https://bio.jmirx.org/1/e68519/

(JMIRx Bio 2025;3:e71627) doi:10.2196/71627

KEYWORDS

ventral pallidum; nucleus accumbens shell; chemogenetics; sucrose; feeding behavior; food motivation; palatable food; DREADD; designer receptors exclusively activated by designer drugs

This is a peer-review report submitted for the paper “Effects of Ventral Pallidum–Nucleus Accumbens Shell Neural Pathway Modulation on Sucrose Consumption and Motivation in Female Rats: Chemogenetic Manipulation Study.”

Round 1 Review

General Comments

The manuscript from Peroutka and Covelo [1] describes the results of chemogenic activation or inhibition of the ventral pallidum–nucleus accumbens shell pathway in adult female rats on sucrose intake (20% sucrose bottle access) versus operant response–provided food pellets delivered on a progressive ratio schedule. The rats were not food restricted. Activation of the pathway decreased sucrose intake while inactivation of the pathway increased sucrose intake. Activation or inactivation did not clearly alter responding for food pellets. The authors provide discussion including an interpretation of the results, such that this pathway is important for sucrose consumption but not motivation for food. This is an interesting study that has some limitations listed below.

Specific Comments

Major Comments

1. Why were only female rats used for this study?

2. What was the approximate age of the rats at the start of the study?

3. The conclusion of the pathway being relevant for sucrose consumption but not food motivation is reasonable, but it would be stronger if the comparisons were made with sucrose consumption versus sucrose motivation and also food consumption versus food motivation.

Minor Comments

4. Are there more objective data from analysis of the immunohistochemistry? What is presented are representative images, but was there any quantification done?

5. The authors discuss cell types but do not specify the likely type of neurons stimulated in this study; is it possible to do so?

Round 2 Review

General Comments

The authors have addressed my concerns from the initial draft.

Conflicts of Interest

None declared.

Reference

1. Peroutka M, Rivero Covelo I. Effects of ventral pallidum–nucleus accumbens shell neural pathway modulation on sucrose consumption and motivation in female rats: chemogenetic manipulation study. JMIRx Bio 2025;3(1):e68519 [FREE Full text] [doi: 10.2196/68519]

Edited by O Singh; submitted 22.01.25; this is a non–peer-reviewed article;accepted 22.01.25; published 08.03.25.

Please cite as:
Grimm J
Peer Review of "Effects of Ventral Pallidum–Nucleus Accumbens Shell Neural Pathway Modulation on Sucrose Consumption and Motivation in Female Rats: Chemogenetic Manipulation Study"
JMIRx Bio 2025;3:e71627
URL: https://bio.jmirx.org/2025/1/e71627
doi:10.2196/71627
PMID:

Peer-Review Report

Peer Review of "Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran: Cross-Sectional Study"

Jiayi Shen[1]

University of Southern California, Los Angeles, CA, United States

Related Articles:

Companion article: https://preprints.jmir.org/preprint/69542

Companion article: https://bio.jmirx.org/2025/1/e72764/

Companion article: https://bio.jmirx.org/2025/1/e69542/

(JMIRx Bio 2025;3:e72765) doi:10.2196/72765

KEYWORDS

impact of climate; seasonal change; frequency; livestock; ticks; Tehran

This is a peer-review submitted for the paper "Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran: Cross-Sectional Study."

Round 1 Review

General Comments

This paper [1] provides a detailed investigation into the distribution and frequency of tick species infecting livestock and poultry in Tehran province, Iran, with a focus on seasonal and climatic variation. The study highlights the significant economic and epidemiological impact of ticks as ectoparasites and pathogen vectors in livestock.

Specific Comments

Major Comments

1. "Material and methods - Sampling": For the tick sampling in this manuscript, what method did you used in this study (ie, how did you decide which tick samples to include and which samples to exclude)? How did you ensure that the sample is representative of the true distribution of ticks in the study area? The distribution and frequency estimates from this sample might not be extended to the whole tick population if the sampling is biased.

2. "Material and methods - Sampling": Could the author provide the rationale or justification of the choice of "*p*" and "*d*" in the sample size calculation?

3. "Discussion": I really like the analysis of seasonal trend presented in Figure 4. Could the author elaborate more on this in the Discussion—the general seasonal trend across all species, the reason why you expect some species to be more abundant in warmer versus colder weather, and the implications from the public health perspective?

Minor Comments

1. Line 41: Spell out "$."

2. Line 44: Remove "(4)"—duplicated reference number.

3. Line 51: Remove "(9)"—duplicated reference number.

4. Line 85, "valid diagnostic keys": Could the author be more specific about the "diagnostic key" being used? Adding a sentence to briefly describe the key would be great.

5. Lines 147-151, "two professional stereo microscopes...in the entomological research": This part should belong to Methods section.

Round 2 Review

Thank you, author, for addressing all my comments and making all necessary changes to the manuscript. I do not have any more comments.

Conflicts of Interest

None declared.

Reference

1. Abbasi E. Assessing the influence of seasonal and climatic variations on livestock tick incidence in Tehran province, Iran: cross-sectional study. JMIRx Bio 2025;3:e69542. [doi: 10.2196/69542]

Edited by J Ren; submitted 17.02.25; this is a non–peer-reviewed article;accepted 17.02.25; published 31.03.25.

Please cite as:
Shen J
Peer Review of "Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran: Cross-Sectional Study"
JMIRx Bio 2025;3:e72765
URL: https://bio.jmirx.org/2025/1/e72765
doi:10.2196/72765
PMID:

Peer-Review Report

Peer Review of "Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran: Cross-Sectional Study"

Vahid Noaman[1]

Razi Vaccine & Serum Research Institute, Karaj, Iran

Related Articles:

Companion article: https://preprints.jmir.org/preprint/69542

Companion article: https://bio.jmirx.org/2025/1/e72764/

Companion article: https://bio.jmirx.org/2025/1/e69542/

(JMIRx Bio 2025;3:e72766) doi:10.2196/72766

KEYWORDS

impact of climate; seasonal change; frequency; livestock; ticks; Tehran

This is a peer-review submitted for the paper "Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran: Cross-Sectional Study."

Round 1 Review

General Comments

The manuscript [1] presents a comprehensive study on the seasonal and climatic distribution of ticks in Tehran province, Iran. The research is well structured and provides valuable insights into the diversity and abundance of tick species in different climate zones. The study is relevant to the field of veterinary parasitology and has potential implications for tick control strategies in the region. However, there are several areas where the manuscript could be improved in terms of grammar, sentence structure, and adherence to standard academic writing conventions.

Specific Comments

Major Comments

Grammar and Sentence Structure

Overall clarity: The manuscript is generally clear, but there are instances where the sentence structure could be improved for better readability. Some sentences are overly long and could be broken down into shorter, more concise statements.

For example:

- Original: "The presence of ticks on livestock causes localized lesions at the bite site and systemic lesions, leading to death due to anemia and paralysis due to ticks transmitting various diseases such as theileriosis and babesiosis."
- Suggested revision: "The presence of ticks on livestock causes localized lesions at the bite site and systemic effects, which can lead to anemia, paralysis, and even death. Ticks are also vectors for diseases such as theileriosis and babesiosis."

Subject-verb agreement: There are a few instances where the subject-verb agreement is incorrect.

For example:

- Original: "The distribution of collected ticks (in mountain and plain climates) indicated that out of 806 collected ticks, 44.78% and 55.21% belonged to the mountainous and plain regions, respectively."
- Suggested revision: "The distribution of collected ticks (in mountainous and plain climates) indicates that out of 806 collected ticks, 44.78% belonged to mountainous regions, while 55.21% were found in plain regions."

Tense consistency: The manuscript occasionally shifts between past and present tense. It is important to maintain consistency, especially in the Results and Discussion sections.

For example:

- Original: "The study is conducted in two different environments: plains and mountains within 20 selected villages in Tehran Province."
- Suggested revision: "The study was conducted in two different environments: plains and mountains within 20 selected villages in Tehran Province."

Structure and Organization

Abstract: The Abstract is well written and provides a concise summary of the study. However, it could benefit from a brief mention of the key findings related to seasonal variations, as this is a major focus of the study.

Introduction: The Introduction provides a good background on the importance of ticks and their impact on livestock. However, it could be strengthened by including more recent references (post-2020) to highlight the current state of research on tick-borne diseases and climate change.

Methods: The Methods section is detailed and well organized. However, the formula used for sample size calculation is not clearly explained. It would be helpful to provide a brief explanation of the variables used in the formula (eg, p=0.3 and d=0.045).

Results: The Results are presented clearly, with appropriate use of tables and figures. However, some of the tables could be simplified for better readability. For example, Table 4 could be restructured to make it easier to compare seasonal activity across species.

Discussion: The Discussion is thorough and compares the findings with other studies effectively. However, it could be improved by discussing the limitations of the study and suggesting areas for future research.

Similarity and Plagiarism

The manuscript appears to be original, with no significant issues of plagiarism detected. However, it is recommended to run the manuscript through a plagiarism detection tool (eg, Turnitin) to ensure that all sources are properly cited and that there is no unintentional duplication of text.

Adherence to Standard Academic Writing

References: The references are generally appropriate and relevant to the study. However, some references are quite old (eg, references from the 1980s and 1990s). It is recommended to include more recent studies to reflect the current state of knowledge in the field.

Add these references to the manuscript:

1. Noaman V. Identification of hard ticks collected from sheep naturally infected with Anaplasma ovis in Isfahan province, central Iran. Comp Clin Pathol 2012 Feb 21; 21(3):367-369. [doi: 10.1007/s00580-012-1438-1]
2. Noaman V, Abdigoudarzi M, Nabinejad AR. Abundance, diversity and seasonal dynamics of hard ticks infesting cattle in Isfahan province, central Iran. Archives of Razi Institute. 2017 Mar 1;72(1):15-21. [doi: 10.22034/ari.2016.107490]
3. Noaman V, Abdigoudarzi M, Nabinejad AR, Heidari MR, Khalilifard M. (2007). Identification of hard ticks of domestic ruminants in two ecological zones of Isfahan province, Iran. Veterinary Journal (Pajouhesh va Sazandegi). 2008;77:88-95.

Figures and tables: The figures and tables are well presented and support the findings of the study. However, the legends for some figures (eg, Figure 1) could be more descriptive. For example, Figure 1 could include a brief explanation of what the "ratio of caught ticks" represents.

The manuscript presents a valuable contribution to the field of veterinary parasitology, particularly in the context of tick distribution and seasonal activity in Tehran province. With some revisions to improve grammar, sentence structure, and adherence to standard academic writing conventions, the manuscript will be suitable for publication in a reputable journal. The manuscript can be considered for publication in *JMIRx Bio* after major revision.

Round 2 Review

General Comments

The revised manuscript titled "Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran" has addressed the previous comments and suggestions effectively. The authors have made the necessary revisions to improve the clarity, structure, and overall quality of the manuscript. Below are my final comments.

Strengths

Improved clarity: The Abstract has been revised to be more concise and now includes key findings related to seasonal variation and the most abundant tick species, enhancing readability and impact.

Focused introduction: The Introduction now more clearly highlights the specific gaps in the literature that this study addresses, particularly in the context of Tehran province.

Streamlined methodology: The Methods section has been clarified, with more details on the randomization process and a more concise description of the study area. The inclusion of supplementary tables for geographical coordinates and ecological information is a welcomed addition.

Organized results: The Results section has been streamlined with the use of subheadings, making it easier to follow. All referenced figures and tables are now included, providing a comprehensive view of the findings.

Enhanced discussion: The Discussion section now more effectively focuses on the implications of the findings for tick control strategies in Tehran province. The comparison with studies from other regions has been made more concise, emphasizing key similarities and differences.

Practical conclusion: The Conclusion has been revised to highlight the practical implications of the findings, particularly the need for seasonal tick control measures in different climate zones.

Consistent references: All references are now formatted consistently according to the journal's guidelines, with complete details provided.

Overall recommendation: The manuscript has been significantly improved and is now suitable for publication. I recommend acceptance of the manuscript in its current form.

Conflicts of Interest

None declared.

Reference

1. Abbasi E. Assessing the influence of seasonal and climatic variations on livestock tick incidence in Tehran province, Iran: cross-sectional study. JMIRx Bio 2025;3:e69542. [doi: 10.2196/69542]

Edited by J Ren; submitted 17.02.25; this is a non–peer-reviewed article;accepted 17.02.25; published 31.03.25.

Please cite as:
Noaman V
Peer Review of "Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran: Cross-Sectional Study"
JMIRx Bio 2025;3:e72766
URL: https://bio.jmirx.org/2025/1/e72766
doi:10.2196/72766
PMID:

Authors' Response to Peer Reviews

Authors' Response to Peer Reviews of "Effects of Ventral Pallidum–Nucleus Accumbens Shell Neural Pathway Modulation on Sucrose Consumption and Motivation in Female Rats: Chemogenetic Manipulation Study"

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Companion article: https://bio.jmirx.org/2025/1/e71626/

Companion article: https://bio.jmirx.org/2025/1/e68519/

(JMIRx Bio 2025;3:e71629) doi:10.2196/71629

KEYWORDS

ventral pallidum; nucleus accumbens shell; chemogenetics; sucrose; feeding behavior; food motivation; palatable food; DREADD; designer receptors exclusively activated by designer drugs

This is the authors' response to peer-review reports for "Effects of Ventral Pallidum–Nucleus Accumbens Shell Neural Pathway Modulation on Sucrose Consumption and Motivation in Female Rats: Chemogenetic Manipulation Study."

Round 1 Review

Reviewer C [1]

General Comments

In this paper [2], the authors present an interesting and well-written paper dealing with the effects of stimulation and inhibition of projections from the ventral pallidum to the nucleus accumbens shell on feeding and food reinforced behaviors. The methods used are cutting edge, and my comments and suggestions are relatively minor.

Minor Comments

1. In the third paragraph of the Introduction, the sentence beginning with "Parallelly" is very awkward; I am sure there is a way to word this that does not use "parallelly." Also, the previous sentence could be made clearer as to whether effects on sucrose consumption are found just in female rats.

Response: The paragraph has been reworded for clarity and to minimize its possible awkwardness. Moreover, we believe the current phrasing emphasizes that the results were observed only in female rats.

2. The number of subjects should be listed in the Methods.

Response: In the original manuscript, the number of subjects was listed in the Methods section under the subsection "Immunohistochemistry." The authors recognize that this is an

unorthodox location for that kind of information, and now, the number of subjects can be found in the "Subjects" subsection.

> *3. In the last paragraph of the body of the manuscript, the sentence beginning with "The discrepancies observed across studies of this pathway..." is unfinished, and I am uncertain what the authors intended to say.*

Response: The offending sentence has been removed from the paragraph. The authors would like to thank the reviewer for the careful reading of the manuscript.

> *4. In discussing the differences between the results observed here and those reported by Vanchez et al [3], is it possible that these may reflect the use of "closed-loop" manipulations linked to the occurrence of licking in the Vanchez et al [3] paper, in contrast to the continuous modulation produced here by the use of the DREADD (designer receptors exclusively activated by designer drugs) technique? Also, in this section, the authors could be a bit clearer as to why the techniques used by Vanchez et al [3] would be expected to label a different subpopulation of cells than was the case in this study.*

Response: This paragraph has been expanded in an attempt to address Reviewer C's comments. The authors believe that the current version of the manuscript offers a more nuanced discussion of our findings and those of Vachez et al [3].

Reviewer Q [4]

General Comments

> *The manuscript from Peroutka and Covelo [1] describes the results of chemogenic activation or inhibition of the ventral pallidum–nucleus accumbens shell pathway in adult female rats on sucrose intake (20% sucrose bottle access) versus operant response–provided food pellets delivered on a progressive ratio schedule. The rats were not food restricted. Activation of the pathway decreased sucrose intake while inactivation of the pathway increased sucrose intake. Activation or inactivation did not clearly alter responding for food pellets. The authors provide discussion including an interpretation of the results, such that this pathway is important for sucrose consumption but not motivation for food. This is an interesting study that has some limitations listed below.*

Specific Comments

Major Comments

> *1. Why were only female rats used for this study?*

Response: Historically, much of behavioral neuroscience research has focused primarily on males, leading to a lack of understanding of female brain function. While this study could have been conducted in male rats, we decided to use female rats to generate more information about the female rat brain. The authors acknowledge that future studies should consider studying male rats to observe if sex is a relevant variable in the observed behaviors.

> *2. What was the approximate age of the rats at the start of the study?*

Response: The age of the rats at the start of the study has been added to the Methods section.

> *3. The conclusion of the pathway being relevant for sucrose consumption but not food motivation is reasonable, but it would be stronger if the comparisons were made with sucrose consumption versus sucrose motivation and also food consumption versus food motivation.*

Response: This study only uses sucrose as a reward, either in the form of sucrose pellets in the case of the progressive ration task, or 20% sucrose solution in the case of the free-access task. The authors recognize that the use of the term "food" throughout the manuscript might have contributed to some confusion as to the nature of the reward used. In this version, we have minimized the generic use of the word "food" and specified that sucrose was used all along. The authors still believe that the chemogenetic manipulations described in the manuscript affected sucrose consumption but not the motivation to work for food.

Minor Comments

> *4. Are there more objective data from analysis of the immunohistochemistry? What is presented are representative images, but was there any quantification done?*

Response: As described in the Methods, immunohistochemistry was studied qualitatively to assess DREADD (designer receptors exclusively activated by designer drugs) expression in the relevant brain areas. The authors consider this analysis to be sufficient to support the conclusions presented in the manuscript. Future studies could be conducted to assess if the number of DREADD-expressing neurons affects the behavioral outcomes observed, although such studies would require a significantly higher number of animals than those used here.

> *5. The authors discuss cell types but do not specify the likely type of neurons stimulated in this study; is it possible to do so?*

Response: The question of the nature of the cells expressing DREADD is interesting and worth studying in the future. Unfortunately, at this time, it is not logistically possible for the authors to conduct such studies.

Round 2 Review

Reviewer Q [4]

General Comments

> *The authors have addressed my concerns from the initial draft.*

References

1. Wirtshafter D. Peer review of "Effects of Ventral Pallidum–Nucleus Accumbens Shell Neural Pathway Modulation on Sucrose Consumption and Motivation in Female Rats: Chemogenetic Manipulation Study". JMIRx Bio 2025;3(1):e71626 [FREE Full text] [doi: 10.2196/71626]
2. Peroutka M, Rivero Covelo I. Effects of ventral pallidum–nucleus accumbens shell neural pathway modulation on sucrose consumption and motivation in female rats: chemogenetic manipulation study. JMIRx Bio 2025;3(1):e68519 [FREE Full text] [doi: 10.2196/68519]
3. Vachez YM, Tooley JR, Abiraman K, Matikainen-Ankney B, Casey E, Earnest T, et al. Ventral arkypallidal neurons inhibit accumbal firing to promote reward consumption. Nat Neurosci 2021 Mar;24(3):379-390 [FREE Full text] [doi: 10.1038/s41593-020-00772-7] [Medline: 33495635]
4. Grimm J. Peer review of "Effects of Ventral Pallidum–Nucleus Accumbens Shell Neural Pathway Modulation on Sucrose Consumption and Motivation in Female Rats: Chemogenetic Manipulation Study". JMIRx Bio 2025;3(1):e71627 [FREE Full text] [doi: 10.2196/71627]

Abbreviations

DREADD: designer receptors exclusively activated by designer drugs

Edited by O Singh; submitted 22.01.25; this is a non–peer-reviewed article;accepted 22.01.25; published 08.03.25.

Please cite as:
Peroutka M, Rivero Covelo I
Authors' Response to Peer Reviews of "Effects of Ventral Pallidum–Nucleus Accumbens Shell Neural Pathway Modulation on Sucrose Consumption and Motivation in Female Rats: Chemogenetic Manipulation Study"
JMIRx Bio 2025;3:e71629
URL: https://bio.jmirx.org/2025/1/e71629
doi:10.2196/71629
PMID:

Author's Response to Peer Reviews

Author's Response to Peer Reviews of "Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran: Cross-Sectional Study"

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Companion article: https://bio.jmirx.org/2025/1/e69542/

(JMIRx Bio 2025;3:e72764) doi:10.2196/72764

KEYWORDS

impact of climate; seasonal change; frequency; livestock; ticks; Tehran

Author's response to peer reviews for "Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran: Cross-Sectional Study."

Round 1 Review

Reviewer W [1]

General Comments

> *This paper provides a detailed investigation into the distribution and frequency of tick species infecting livestock and poultry in Tehran province, Iran, with a focus on seasonal and climatic variation. The study highlights the significant economic and epidemiological impact of ticks as ectoparasites and pathogen vectors in livestock.*

Response: We sincerely appreciate the time and effort you have taken to review our manuscript [2]. Your feedback is valuable, and we have addressed all of your comments as outlined below.

Specific Comments

Major Comments

> *1. "Material and methods - Sampling": For the tick sampling in this manuscript, what method did you used in this study (ie, how did you decide which tick samples to include and which samples to exclude)? How did you ensure that the sample is representative of the true distribution of ticks in the study area? The distribution and frequency estimates from this sample might not be extended to the whole tick population if the sampling is biased.*

Response: In this study, tick samples were collected from 1623 livestock animals (including chickens, camels, cows, pigeons, dogs, and sheep) infected with ticks. The sampling was carried

out using a cross-sectional study design in two different climate regions: mountainous and plain. Livestock were randomly selected based on visible tick infestation, ensuring that the sample represented the true distribution of ticks in the study area, particularly with the help of local veterinary authorities.

Text to be added to the Sampling section of the Methods: "To ensure representative sampling, a cross-sectional study was conducted, covering both mountainous and plain regions. Selection of livestock was randomized among those showing visible tick infestation, with veterinary supervision ensuring consistency in sample collection across different geographical zones. The chosen method aligns with established epidemiological studies on tick distribution."

2. "Material and methods - Sampling": Could the author provide the rationale or justification of the choice of "p" and "d" in the sample size calculation?

Response: The parameter p=0.3 was selected based on previous studies on tick prevalence in similar regions of Iran, where an approximate prevalence rate of 30% was observed. The margin of error (d=0.045) was chosen to ensure a 95% confidence level while maintaining a practical sample size for data collection.

Text to be added to the manuscript: "The parameter *P* (0.3) was selected based on prior studies on tick prevalence in similar regions in Iran, indicating an estimated infestation rate of 30%. The margin of error (d = 0.045) was determined considering a 95% confidence level, ensuring a balance between precision and feasibility of sample collection."

3. "Discussion": I really like the analysis of seasonal trend presented in Table 4 and Figure 4. Could the author elaborate more on this in the Discussion—the general seasonal trend across all species, the reason why you expect some species to be more abundant in warmer versus colder weather, and the implications from the public health perspective?

Response: The analysis revealed that hard ticks like *Rhipicephalus sanguineus* and *Hyalomma marginatum* were more abundant during the spring and summer, likely due to higher temperatures and increased activity of their hosts. In contrast, soft ticks such as *Argas persicus* were more prevalent in the autumn and winter, as they can survive in sheltered environments and colder conditions.

Text to be added to the Discussion section: "The observed seasonal trend aligns with the biological cycles of tick species. Hard ticks such as *Rhipicephalus sanguineus* and *Hyalomma marginatum* exhibited peak abundance in spring and summer due to higher temperatures and increased host activity. Conversely, soft ticks (*Argas persicus*) showed resilience during colder months, likely due to their ability to survive in sheltered environments. This seasonal variability highlights the importance of targeted tick control strategies, particularly in warm seasons when transmission risk of tick-borne diseases is highest."

Minor Comments

1. Line 41: Spell out "$."

2. Line 44: Remove "(4)"—duplicated reference number.

3. Line 51: Remove "(9)"—duplicated reference number.

4. Line 85, "valid diagnostic keys": Could the author be more specific about the "diagnostic key" being used? Adding a sentence to briefly describe the key would be great.

Response: To clarify the "valid diagnostic key" used for tick identification, we will specify the exact key used for species identification and provide a brief description of its methodology.

Text to be added to the manuscript (Line 85 of Methods): "The tick species were identified using the diagnostic keys outlined by Jongejan et al. (1987) [3] and Camicas et al. (1998) [4], which provide detailed morphological descriptions and illustrations for the identification of both soft and hard ticks. These keys are widely recognized for their accuracy and reliability in the identification of tick species in the Middle East and neighboring regions."

5. Lines 147-151, "two professional stereo microscopes...in the entomological research": This part should belong to Methods section.

Reviewer AX [5]

General Comments

The manuscript [1] presents a comprehensive study on the seasonal and climatic distribution of ticks in Tehran province, Iran. The research is well structured and provides valuable insights into the diversity and abundance of tick species in different climate zones. The study is relevant to the field of veterinary parasitology and has potential implications for tick control strategies in the region. However, there are several areas where the manuscript could be improved in terms of grammar, sentence structure, and adherence to standard academic writing conventions.

Specific Comments

Major Comments

Grammar and Sentence Structure

Overall clarity: The manuscript is generally clear, but there are instances where the sentence structure could be improved for better readability. Some sentences are overly long and could be broken down into shorter, more concise statements.

For example:

- *Original: "The presence of ticks on livestock causes localized lesions at the bite site and systemic lesions, leading to death due to anemia and paralysis due to ticks transmitting various diseases such as theileriosis and babesiosis."*
- *Suggested revision: "The presence of ticks on livestock causes localized lesions at the bite site and systemic effects, which can lead to anemia, paralysis, and even death. Ticks*

are also vectors for diseases such as theileriosis and babesiosis."

Subject-verb agreement: There are a few instances where the subject-verb agreement is incorrect.

For example:

- *Original: "The distribution of collected ticks (in mountain and plain climates) indicated that out of 806 collected ticks, 44.78% and 55.21% belonged to the mountainous and plain regions, respectively."*
- *Suggested revision: "The distribution of collected ticks (in mountainous and plain climates) indicates that out of 806 collected ticks, 44.78% belonged to mountainous regions, while 55.21% were found in plain regions."*

Tense consistency: The manuscript occasionally shifts between past and present tense. It is important to maintain consistency, especially in the Results and Discussion sections.

For example:

- *Original: "The study is conducted in two different environments: plains and mountains within 20 selected villages in Tehran Province."*
- *Suggested revision: "The study was conducted in two different environments: plains and mountains within 20 selected villages in Tehran Province."*

Response: We agree, and several sentences will be broken down for better clarity and conciseness. Below is an example of a revised sentence:

- Original: "The presence of ticks on livestock causes localized lesions at the bite site and systemic lesions, leading to death due to anemia and paralysis due to ticks transmitting various diseases such as theileriosis and babesiosis."
- Revised: "Ticks on livestock cause localized bite-site lesions and systemic effects. They can lead to anemia, paralysis, and even death by transmitting diseases like theileriosis and babesiosis."

Structure and Organization

Abstract: The Abstract is well written and provides a concise summary of the study. However, it could benefit from a brief mention of the key findings related to seasonal variations, as this is a major focus of the study.

Response: Yes, the Abstract will be revised to include more specific quantitative data, such as sample size, species abundance, and seasonal variations.

Revised Abstract (Results section): "Results showed that out of 806 collected ticks, 44.78% were found in mountainous regions and 55.21% in plain regions. The most abundant species was *Rhipicephalus sanguineus* (36.97%), while *Rhipicephalus (Boophilus) annulatus* was the least common (0.37%). Seasonal variation indicated peak infestation in spring (60.3%) and lowest in winter (9.5%)."

Introduction: The Introduction provides a good background on the importance of ticks and their impact on livestock. However, it could be strengthened by including more recent references (post-2020) to highlight the current state of research on tick-borne diseases and climate change.

Methods: The Methods section is detailed and well organized. However, the formula used for sample size calculation is not clearly explained. It would be helpful to provide a brief explanation of the variables used in the formula (eg, p=0.3 and d=0.045).

Response: A brief explanation of the sample size calculation formula will be added to the Methods section for clarity.

Text to be added to the Sample Size Calculation section of the Methods: "The sample size was calculated using Cochran's formula for prevalence studies. Given an estimated prevalence (*p*) of 30% and a precision (*d*) of 4.5%, the final sample size was determined to be 800 ticks, ensuring statistical reliability."

Results: The Results are presented clearly, with appropriate use of tables and figures. However, some of the tables could be simplified for better readability. For example, Table 4 could be restructured to make it easier to compare seasonal activity across species.

Discussion: The Discussion is thorough and compares the findings with other studies effectively. However, it could be improved by discussing the limitations of the study and suggesting areas for future research.

Similarity and Plagiarism

The manuscript appears to be original, with no significant issues of plagiarism detected. However, it is recommended to run the manuscript through a plagiarism detection tool (eg, Turnitin) to ensure that all sources are properly cited and that there is no unintentional duplication of text.

Adherence to Standard Academic Writing

References: The references are generally appropriate and relevant to the study. However, some references are quite old (eg, references from the 1980s and 1990s). It is recommended to include more recent studies to reflect the current state of knowledge in the field.

Add these references to the manuscript:

1. *Noaman V. Identification of hard ticks collected from sheep naturally infected with Anaplasma ovis in Isfahan province, central Iran. Comp Clin Pathol 2012 Feb 21; 21(3):367-369. [doi: 10.1007/s00580-012-1438-1]*
2. *Noaman V, Abdigoudarzi M, Nabinejad AR. Abundance, diversity and seasonal dynamics of hard ticks infesting cattle in Isfahan province, central Iran. Archives of Razi Institute. 2017 Mar 1;72(1):15-21. [doi: 10.22034/ari.2016.107490]*
3. *Noaman V, Abdigoudarzi M, Nabinejad AR, Heidari MR, Khalilifard M. (2007). Identification of hard ticks of domestic ruminants in two ecological zones of Isfahan province, Iran. Veterinary Journal (Pajouhesh va Sazandegi). 2008;77:88-95.*

Response: The following recent references will be added to the manuscript:

1. Noaman V. Identification of hard ticks collected from sheep naturally infected with Anaplasma ovis in Isfahan province, central Iran. Comp Clin Pathol 2012 Feb 21; 21(3):367-369. [doi: 10.1007/s00580-012-1438-1]
2. Noaman V, Abdigoudarzi M, Nabinejad AR. Abundance, diversity and seasonal dynamics of hard ticks infesting cattle in Isfahan province, central Iran. Archives of Razi Institute. 2017 Mar 1;72(1):15-21. [doi: 10.22034/ari.2016.107490]
3. Noaman V, Abdigoudarzi M, Nabinejad AR, Heidari MR, Khalilifard M. (2007). Identification of hard ticks of domestic ruminants in two ecological zones of Isfahan province, Iran. Veterinary Journal (Pajouhesh va Sazandegi). 2008;77:88-95.

Text to be added to the Discussion section: “Our findings align with previous studies on tick diversity in central Iran (Noaman et al., 2012; Noaman et al., 2017), confirming seasonal variations in tick populations. These studies further support the need for region-specific tick control strategies.”

> *Figures and tables: The figures and tables are well presented and support the findings of the study. However, the legends for some figures (eg, Figure 1>) could be more descriptive. For example, Figure 1 could include a brief explanation of what the “ratio of caught ticks” represents.*
>
> *The manuscript presents a valuable contribution to the field of veterinary parasitology, particularly in the context of tick distribution and seasonal activity in Tehran province. With some revisions to improve grammar, sentence structure, and adherence to standard academic writing conventions, the manuscript will be suitable for publication in a reputable journal. The manuscript can be considered for publication in JMIRx Bio after major revision.*

Response: We trust that these revisions adequately address the reviewer’s concerns. Please let us know if any further modifications are required. We look forward to your feedback on the revised manuscript.

Round 2 Review

Reviewer W

> *Thank you, author, for addressing all my comments and making all necessary changes to the manuscript. I do not have any more comments.*

Reviewer AX

General Comments

> *The revised manuscript titled “Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran” has addressed the previous comments and suggestions effectively. The authors have made the necessary revisions to improve the clarity, structure, and overall quality of the manuscript. Below are my final comments.*

Strengths

> *Improved clarity: The Abstract has been revised to be more concise and now includes key findings related to seasonal variation and the most abundant tick species, enhancing readability and impact.*
>
> *Focused introduction: The Introduction now more clearly highlights the specific gaps in the literature that this study addresses, particularly in the context of Tehran province.*
>
> *Streamlined methodology: The Methods section has been clarified, with more details on the randomization process and a more concise description of the study area. The inclusion of supplementary tables for geographical coordinates and ecological information is a welcomed addition.*
>
> *Organized results: The Results section has been streamlined with the use of subheadings, making it easier to follow. All referenced figures and tables are now included, providing a comprehensive view of the findings.*
>
> *Enhanced discussion: The Discussion section now more effectively focuses on the implications of the findings for tick control strategies in Tehran province. The comparison with studies from other regions has been made more concise, emphasizing key similarities and differences.*
>
> *Practical conclusion: The Conclusion has been revised to highlight the practical implications of the findings, particularly the need for seasonal tick control measures in different climate zones.*
>
> *Consistent references: All references are now formatted consistently according to the journal’s guidelines, with complete details provided.*
>
> *Overall recommendation: The manuscript has been significantly improved and is now suitable for publication. I recommend acceptance of the manuscript in its current form.*

Conflicts of Interest

None declared.

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1. Shen J. Peer review of “Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran: Cross-Sectional Study”. JMIRx Bio 2025;3:e72765. [doi: 10.2196/72765]

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4. Camicas JL, Hervy JP, Adam F, Morel PC. Les Tiques du Monde (Acarida, Ixodida): Nomenclature, Stades Décrits, Hôtes, Répartition. The Ticks of The World (Acarida, Ixodida): Nomenclature, Described Stages, Hosts, Distribution. Paris, France: Éditions de l'Orstom; 1998.
5. Noaman V. Peer review of "Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran: Cross-Sectional Study". JMIRx Bio 2025;3:e72764. [doi: 10.2196/72766]

Edited by J Ren; submitted 17.02.25; this is a non–peer-reviewed article;accepted 17.02.25; published 31.03.25.

Please cite as:
Abbasi E
Author's Response to Peer Reviews of "Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran: Cross-Sectional Study"
JMIRx Bio 2025;3:e72764
URL: https://bio.jmirx.org/2025/1/e72764
doi:10.2196/72764
PMID:

Publisher:
JMIR Publications
130 Queens Quay East.
Toronto, ON, M5A 3Y5
Phone: (+1) 416-583-2040
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