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Exploring the possible link between toxoplasmosis and crib-biting behavior in horse farms of Shiraz: a case, control study

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Abstract

Toxoplasma is an intracellular parasite that can cause toxoplasmosis in humans and animals. In this disease, parasitic cysts of *Toxoplasma gondii* can remain present in various tissues such as the brain throughout the host's life. The potential role of toxoplasmosis should be considered in many neurological diseases with unknown mechanisms. In horses, behavioral disorders like crib-biting have multifactorial causes and may be due to neurophysiological dysfunction. This research aimed to determine the role of toxoplasmosis in the manifestation of crib-biting behavior in horses. A case-control study was conducted in horse riding clubs in Fars province, Iran, near the city of Shiraz. Ten horses with crib-biting behavior and 10 clinically healthy horses matched for sex, age, and breed were enrolled in the study. Blood samples were collected from cribbers and healthy horses, along with a thorough history-taking. An enzyme-linked immunosorbent assay (ELISA) was performed on sera to detect *T. gondii*-specific Immunoglobulin G (IgG) and Immunoglobulin M (IgM). The results of this study revealed that all horses were free of toxoplasmosis. It is concluded that this relation was not detected in the horse farms of Shiraz suburbs. Further research is required to explore more aspects of crib-biting behavior and its relationship with causative factors.

Introduction

Toxoplasmosis is a common parasitic zoonosis found in humans and other warm-blooded vertebrates worldwide. *Toxoplasma gondii* is a coccidian protozoan capable of infecting a wide range of hosts and host cells through various routes of transmission (1). Infection by this protozoan results in the formation of parasite cysts that persist in several tissues, primarily in neural and muscular tissues including the brain, eyes, and skeletal and cardiac muscles throughout the host's life (2, 3). *T. gondii* shows a strong affinity for brain tissue (4), and the fundamental aspects of the interactions between the protozoan and the host are still largely unknown (5). A chronic infection state is established by *T. gondii* in the brain and skeletal muscle of its mammalian host. The blood-brain barrier is crossed by Toxoplasma as either

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extracellular tachyzoites that infect and replicate with brain endothelial cells or within an infected monocyte, but there is limited understanding of how Toxoplasma infections of the brain and skeletal muscle and the resulting inflammation impact the tissues' function. Recent studies have revealed that both excitatory and inhibitory neurotransmission in the central nervous system are altered by Toxoplasma, and these changes lead to unbalanced synaptic activity and seizures (6). Therefore, the potential role of toxoplasmosis should be considered in many related diseases with unknown mechanisms. The behavior of hosts can be altered and the risk of predation and transmission to other hosts is potentially increased by *T. gondii*. In rodents, infection has been associated with a reduction in innate fear and an increase in risk-taking behavior toward cat odor. In foxes, a similar phenomenon known as Dopey Fox Syndrome has been observed, with abnormal behavior such as a lack of fear towards humans and an increase in daytime activity exhibited by infected animals. Toxoplasma infection may be a contributing factor to this behavior, potentially manipulating the host's immune system and altering neurotransmitter levels in the brain, although the precise mechanisms are not fully understood (7).

The seroprevalence of *T. gondii* in horses has been studied in various regions, including Iran (8), Turkey (9), Saudi Arabia (10), China (11), and Brazil (12). These studies highlight the global distribution of toxoplasmosis in equine populations. Additionally, the detection of anti-*T. gondii* antibodies in horses underscores the importance of understanding the prevalence and potential implications of this parasitic infection in horses. Crib biting, a stereotypic oral-based behavior characterized by grasping a solid object with incisor teeth and inhaling air with an audible grunt (13), is associated with altered neurophysiological function (14, 15) and is similarly associated with brain dysfunction in schizophrenia, autism, and caged parrots (16). While poor welfare and suboptimal management may contribute to the appearance of crib-biting, the mechanisms of formation and causes of equine stereotypy remain largely speculative (17). In a mouse model of chronic *T. gondii* infection, neurologic and behavioral abnormalities secondary to inflammation and loss of brain parenchyma were observed by Hermes et al. (18).

Yolken et al. (19) reported significantly increased levels of anti-Toxoplasma antibodies in individuals with a first episode of schizophrenia compared with control subjects, suggesting a possible relationship between Toxoplasma infection and the occurrence of schizophrenia. The diagnosis of toxoplasmosis is typically made by detecting immunoglobulins G and M (IgG and IgM) antibodies in serum samples using various methods, including enzyme-linked immunosorbent assay (ELISA), enzyme-linked fluorescence assay (ELFA), immunosorbent agglutination assay (ISAGA), and IgM-indirect fluorescent-antibody (IgM-IFA) (20). IgG antibodies are typically detected within one to two weeks after infection acquisition and persist throughout the host's life, while IgM antibodies may appear earlier but decline more rapidly (21). While the studies provide valuable information on crib-biting behavior and toxoplasmosis in horses, further research is needed to establish a definitive link between these two conditions. Given the worldwide importance of *T. gondii* and the limited data on the correlation between these parasites and certain diseases, this study aimed to examine the potential relationship between toxoplasmosis and crib-biting behavior in the horse farms of Shiraz suburbs.

Materials and Methods

This study was conducted Shiraz suburb, Fars province, south of Iran, around the city of Shiraz with the approval of the State Committee on Animal Ethics at Shiraz University, which followed the guidelines for the protection of animals used for experimental purposes outlined in the European Council Directive 86/609/EC (1986). The study included a total of ten established crib-biting horses, consisting of 7 stallions, 2 mares, and 1 gelding, of

different breeds (including crossbreds, Arabian, Turkmen, and Dareshouri), ranging in age from 2 to 14 years old. The horse underwent an examination based on a history of over a year of involvement in crib-biting disease. The symptoms and signs of crib-biting disease were identified and implemented as per Omidi et al. (22). The horses were housed individually in conventional horse boxes located in various riding stables in the vicinity of Shiraz. The owners reported that the crib-biting behavior had been ongoing for at least one year, although the full history of the behavior was unclear for all of the horses. A control group of ten age- and sex-matched horses with no history of stereotypic behavior, and kept under the same housing conditions, were also included in the study. All horses, including crib biters and controls, received similar feeding, with concentrate included in their daily ration. All blood sampling was performed by a qualified veterinarian. In this study, 10 mL blood samples were collected from the jugular vein of horses, and sera were harvested by centrifuging the blood samples at 750*g* for 15 minutes at room temperature. The sera were stored at -80°C until tested. The anti-*T. gondii*-specific IgM and IgG serum antibodies were analyzed by ELISA using a commercially available kit (Euroimmun, Germany), which included standard positive and negative controls provided in the kits. Antibody levels >10 IU/mL were considered positive.

Results

The serum samples were analyzed for *T. gondii* IgM and IgG antibodies using the ELISA kit. The optical densities (OD) of the samples were measured at 450 nm, and the OD value of the blank well was used to correct all OD readings from the test wells. For IgM, the OD value of the negative control and the cutoff value, after subtracting the blank absorbance, should be <0.2 and 0.2~0.45, respectively. The cutoff value was calculated using the formula: Cut-off value (IgM) = Absorbance of cut-off calibrator 1 - Blank Absorbance.

For IgG, the OD value of the negative control and the cutoff value were <0.9 and 0.9~1.1, respectively, and the cutoff value was calculated using the formula:

Cut off value (IgG) = Absorbance of calibrator 2 - Blank Absorbance.

Samples with OD values equal to or greater than the cutoff value were considered positive for both IgG and IgM antibodies, while samples with OD values less than the cutoff value were considered negative. In this study, all of the tested samples were negative for both IgM and IgG antibodies, indicating no evidence of *T. gondii* infection in the horses studied. Vital signs and characteristics of the horses under study are presented in Tables number 1 and 2.

Table 1. Vital signs and characteristics of horses affected by crib-biting

Number	Gender	Breed	Age (Years)	Heart rate (bpm)	Temperature (°C)	Breathing (bpm)
1	Male	Crossbred	14	30	37	10
2	Male	Thoroughbred-Turkmen	12	34	37.4	12
3	Male	Thoroughbred-Turkmen	12	29	37	15
4	Female	Arabian	9	38	36.3	19
5	Male	Dareshouri	6	31	37.9	20
6	Female	Dareshouri	3	36	37.4	19
7	Male	Turkmen	7	36	37.1	14
8	Male	Arabian	2	48	37.1	24
9	Male	Crossbred	10	32	36.6	20
10	Male	Crossbred	9	36	35.8	24
Mean ± SD			8.4±3.93	35±5.46	36.96±0.6	17.7±4.79

Table 2. Vital signs and characteristics of healthy horses with gender, breed, and age similar to those affected by crib-biting and kept in similar management conditions

Number	Gender	Breed	Age (Years)	Heart rate (bpm)	Temperature (°C)	Breathing (bpm)
1	Male	Crossbred	10	26	36.9	18
2	Male	Thoroughbred-Turkmen	12	32	37.4	15
3	Male	Thoroughbred-Turkmen	12	34	37.7	16
4	Female	Arabian	9	30	36.7	18
5	Male	Dareshouri	8	31	37.3	25
6	Female	Dareshouri	3	32	36.9	20
7	Male	Turkmen	7	37	36.9	20
8	Male	Arabian	2	48	37.1	21
9	Male	Crossbred	10	36	35	20
10	Male	Crossbred	9	36	35.8	24
Mean ± SD			8.2±3.4	34.2±5.87	36.77±0.81	19.7±3.17

Discussion

Toxoplasmosis is a parasitic infection caused by the protozoan parasite *T. gondii*. The infection can affect both humans and animals and is widespread globally (23). In horses, toxoplasmosis is typically a subclinical or latent infection, but it can cause severe illness in pregnant mares and young foals. While the symptoms of toxoplasmosis in horses are generally mild, the infection can have more severe effects on other species, such as cats, where it can cause severe neurological symptoms (24). Detection of *T. gondii* antibodies is the main way to diagnose the infection. The results of the study indicate that the serum IgG and IgM antibodies against *T. gondii* were at low levels in horses with crib-biting behavior, suggesting that this relation was not detected in the horse farms of Shiraz suburbs. The vital signs and characteristics of healthy horses were similar to those affected by crib-biting, and statistically, there was no difference between them. The formation of *T. gondii* cysts in the brain can result in various changes in brain function, including alterations in anatomy, pathology, immunity, neurotransmitters, and gene expression (25). A wide range of behavioral changes in humans and animal models have been attributed to toxoplasmosis. While some studies suggest a possible association between toxoplasmosis and certain diseases such as schizophrenia, depression, anxiety, Alzheimer's disease, Parkinson's disease, and epilepsy, the direction of causality remains controversial and requires further investigation (26). Despite some studies supporting a relationship between toxoplasmosis and behavioral changes in mice models and other animals, this study did not find any evidence of *T. gondii* infection in horses with crib-biting behavior. The relationship between toxoplasmosis and behavioral changes in humans and animals is a controversial area of research. Some studies have suggested a possible link between toxoplasmosis and changes in behavior. However, the direction of causality is not clear, and it is not yet clear whether toxoplasmosis is the cause of these behavioral changes or whether other factors are responsible (27). While it may seem that a sample size of ten affected horses is small for comparison, it is important to note that the disease prevalence is low. The ten horses were selected from various stables that had confirmed crib-biting behavior for at least one year, and a similar horse in terms of age, gender, and breed was chosen for

comparison. Therefore, the results appear to be reliable and accurate at least for the Shiraz region. Nevertheless, crib-biting disease in horses still has an unclear etiology, and such research with various hypotheses will help us get closer to the primary causative factors of the disease. Understanding the underlying causes of stereotypic behaviors in horses is important for developing effective prevention and treatment strategies.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Conflicts of interest

The authors declare that they have no conflict of interest in sampling, analyses, and interpretation of data.

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