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INVESTIGATION OF *NEOSPORA HUGHESI* ANTIBODIES BY USING ELISA IN HORSES IN NINEVEH PROVINCE

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ABSTRACT

The present study have been done from September 2013 to February 2014, on 90 horses of both sex and age at three different regions of Nineveh province - Iraq. Blood samples were collected from jugular vein of 90 horses, some animals appeared clinically healthy while others suffered from different clinical signs. Sera were tested for *Neopora hughesi* antibodies by Recombinant SAG1 Enzyme Linked Immunosorbent Assay ten horses out of 90 (11.11%) reacted positively to *N.hughesi* antibodies. The animal aged 6-10 years recorded high prevalence and suffered from different clinical signs. The antibodies detected in male were more than that of female without any statistically significant.

Key words: *Neospora hughesi, rNh ELISA, SAG1, horses, Nineveh, Iraq.*

INTRODUCTION

Neospora hughesi are apicomplexan protozoa was first described in the brain and spinal cord of an adult horse in California, USA (Marsh *et al*., 1998) *N.hughesi* cause equine protozoal myeloencephalitis (EPM). This neurological disease has been estimated to affect about 1 in 1000 horses annually , the animal suffer from weight loss, head tilting, circling, anorexic, disorientation and symmetric muscle atrophy, the spasticity would increase when the gelding negotiated steps or changes in footing, (Nahms, 2001). The definitive host for *N.hughesi* is not known, the evident of *N.hughesi* has a wider geographic distribution since seropostive horses have been reported in the United State of America, Europe, Asia, and New Zealand (Bartova *et al*., 2010; Duarte *et al*., 2004; Pitel *et al*., 2001).

A treatment protocol for *N.hughesi* has not been described, *N.hughesi* can form tissue cysts and it has been suggested that because of this tissue cyst stage, the parasite would remain refractory to treatment (Carrie *et al*., 2007).

However, different serological methods were used to diagnose *N.hughesi* antibodies in horses (rNh SAG1 ELISA, IFAT, Agglutination test) with differences in sensitivity and specifity (Hoaen *et al*., 2005).

MATERIALS and METHODS

Blood collection and preparation

Blood samples were collected from 90 horses from both sex, different ages, from 3 localities in Nineveh province, Iraq, some animals clinically healthy and others suffer from ataxia in thoracic and pelvic limbs, ataxia appeared relatively symmetric, weight loss, symmetric muscle atrophy was noted in epaxial, gluteal, semimembranosus and semitendinosus muscles, the left pelvic limb was swollen distal to the tarsus. (Figure 1).

Blood samples (5-10ml) were collected from the jugular vein of each horse in vacuum tubes without anticoagulant, the blood samples were transported to the Central laboratory, College of Veterinary medicine, Mosul University, after clotting, samples were centrifuged at 3000 rpm, 10 minutes, serum was decanted and stored at -30^{o}C until tested with rELISA test for the antibodies to *N hughesi*.

Recombinant ELISA

The rELISA was used for detection of antibodies against *N hughesi* in serum, recombinant antigen SAG1 from the equine *N. hughesi* (rNhSAG1) was chosen for use in this test (ATTC), analysis by standard techniques (Andrea *et al*., 2002) the plates were coated with antigen diluted to a final concentration 1ug/ml and later blocked with assay buffer containing 10% dimethyle sulfoxide (DMSO) (SIGMA-ALDRICH), serum samples and peroxidase –conjugated rabbit anti-equine IgG (THERMO-SCIENTIFIC) were diluted 1:100 in DMSO buffer, after incubation and washing the plate were developed, the reaction was stopped and the optical density was determined at wavelength of 450nm with a reference of 650nm using microplate reader to remove interpolate variation a percent positivity (PP) relative to the controls was determined for each test sample, A (PP) was used cut-off of 20%, each sample, control positive and negative (UniProtKB) were run in duplicate on coated wells.

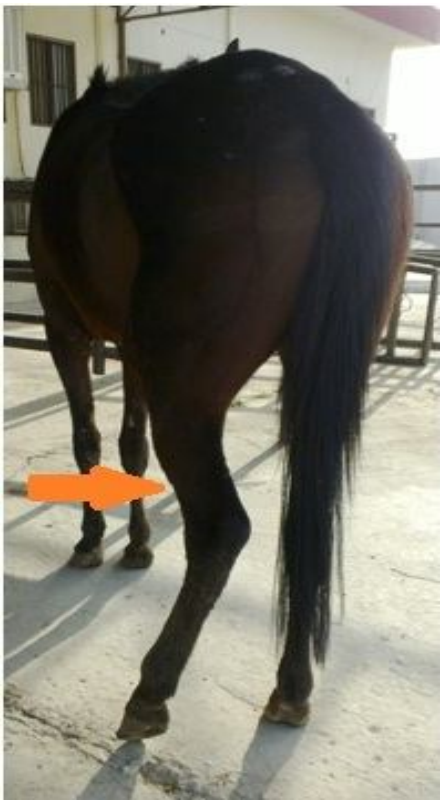

Figure 1: Horse showed ataxia and muscle atrophy of hind limb

RESULTS

Antibodies to *N.hughesi* were detected in 10 horses of 90 serum samples (11.11%) based on rNhSAG1 ELISA analysis (table) the animal aged 6-10 years recorded high prevalence of antibodies, while the antibodies were not detected in animal aged 4-12 months of age (table 1).

According to the sex the male recorded high prevalence with antibodies and was not statistically significant to the percentage of infection in female.

The animal which suffer from different clinical signs showed high prevalence of antibodies than the healthy one.

The animal which suffer from muscle atrophy recorded high seroprevalence when comparing with other clinical signs which animal suffer from it (Table 2).

Table1: General characteristics of horses and seroprevalence of *N hughesi* using rNh SAG1 ELISA.

Characteristics		No. of horses tested	N0. Seropostive *N.hughesi*	Percentage of infection %
Age	4-12months	8	0	0
	2-5years	27	3	11.11
	6-10years	43	6	13.95
	11-17years	12	1	8.3
Sex	Male	49	6	12.24
	Female	41	4	9.76

Table 2: Relationship between health status and seroprevalence of *N hughesi* using rNh SAG1 ELISA

Health status	No.of horses tested	N0.Seropostive *N.hughesi*	Percentage of infection %
Healthy	81	3	3.7
Weight loss	3	2	66.67
Ataxia	4	3	75
Muscle atrophy	2	2	100

DISCUSION

This study is the first reported *N hughesi* seroprevalence study in horses in Iraq, thus the results can be compared with other studies in different countries. In Brasil it was found that 2.6 % of horses had *Neospora* antibodies using IFAT (Anderson *et al*., 2013). In Mexico, USA, 3% of horses had *N.hughesi* antibodies, and no antibodies were detected in foal aged 4-11 months by using cELISA (Michelle *et al*., 2013). In California, 4% of horses were positive to *N.hughesi* antibodies using IFAT and prevalence of antibodies against *N.hughesi* increase with age without detectable risk of trans placental transmission (Duarte *et al*., 2004). In Virginiana, USA 2% horses had *Neospora hughesi* antibodies using IFAT (Vardeleon *et al*., 2001). In USA antibodies of *Neospora* was detected in 3.4 % horses by using rNhSAG1 ELISA (Hoane *et al*., 2004).

Sensitivity and specificity exhibited by the rNhSAG1 ELISA suggest that it has a potential use for serodiagnosis of *N.hughesi* infection in equine when compared with other serological test (Hoane *et al*., 2005).

The animal which suffer from different clinical signs and suffer from muscle atrophy records high prevalence, this result consistent to the researchers (Carrie *et al*., 2007) which reported that the *N.hughesi* in 3 horses which examined clinically and serology, the clinical signs in the 3 horses suffer from ataxia, loss of body weight, muscle atrophy, head tilting, and these researchers give rise to infection with *N.hughesi.*

In conclusion, in this study we found that the *N.hughesi* antibodies have relatively high prevalence when compared this prevalence with other sero prevalence studies in other countries, because no case to use anti-protozoal drugs used in treatment of the horses furthermore no vaccine used.

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التقصي عن اضداد البوغة الخيلية باستخدام المقايسة المناعية المرتبطة بالانزيم في خيول محافظة نينوى

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تمت هذه الدراسة في الفترة من ايلول ٢٠١٣ لغاية شباط ٢٠١٤ ومن ثلاث مناطق مختلفة في محافظة نينوى –العراق، جمعت ٩٠ عينة من الخيول لكلا الجنسين وباعمار متباينة فضلا عن ذلك فان البعض من هذه الحيوانات كانت سليمة ظاهريا في حين كان البعض الاخر منها يعاني من اعراض سريرية متباينة، حيث جمعت عينات الدم من الوريد الوداجي ومن ثم تم فحص المصل لغرض التقصي عن اضداد البوغة الخيلية وباستخدام تقنية المقايسة المناعية المرتبطة بالانزيم والحاوية على المستضد السطحي المتاشب للبوغة الخيلية ، بينت النتائج وجود ١٠ خيول اعطت نتيجة موجبة وبنسبة اصابة كلية (١١.١١%) فضلا عن ذلك فان الحيوانات التي بلغت اعمارها (٦-١٠ سنوات) والحيوانات التي اظهرت اعراض سريرية قد سجلا اعلى نسبة للاصابة، وايضا سجلت نسبة اصابة في الذكور اعلى مما هو عليه في الاناث من غير وجود فروق معنوية.

الكلمات المفتاحية: البوغة الخيلية ، تقنية المقايسة المناعية المرتبطة بالانزيم الحاوية على المستضد السطحي المتاشب للبوغة الخيلية ، الخيول، نينوى ، العراق.