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Seroprevalence of *Neospora caninum* and Some Hematological and Biochemical Parameters in Sheep, Mosul-Iraq

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THE aim of the present study was to determine seroprevalance of *Neosporacanniunm* in serum of sheep by using different serological tests; rapid test and indirect Enzyme Linked Immuno Sorbent Assay (iELISA) in addition to study the changes in some hematological and biochemical parameters accompanied with *Neosporacaninum* in sheep.

Methods : Rapid test and indirect ELISA inthe addition to study of changes in some hematology,and biochemical parameters accompanied with *Neosporacaninum* in sheep.

Results: The study included an examination of 133 blood samples in different areas of Mosul city. Results showed that the prevalence of IgG of Neosporosis by using rapid test and iELISA were 18.04% and 21.05% respectively. Hematology assessment showed no changes in Total Red Blood Cells, Hemoglobin, Packet Cell Volume (PCV), but significant increase in Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC). In contrast it shows a significant increase in Total White Blood Cells in comparison with control. There was a significant increase in AST, ALT, CRP and Cholinesterase. All these parameters examined on seropositive sera for rapid test and iELISA in compared with seronegative .

Conclusion: Presence of *Neosporacaninum* in sheep in Mosul cityare diagnosed by both applied diagnostic methods, fast test and iELISA and gave a good result, in addition, to the detection of IgG, hematological and biochemical parameters in the infected sheep.

Keywords: *Neospora caninum,* Fast test, Indirect Enzyme linked immunosorbent assay, Sheep.

Introduction

Neosporosis is regarded as one of the important diseases discovered in the last years that cause several problems to animals. It is a parasitic disease caused by *Neosporacaninum* an apicomplexhetroxeous parasite that parasite on animals' bodies to complete life cycle.

The importance of the disease came from the economic losses for humans beings when animals became sick further it affect many systems, especially the genital system of the ovine, and causes many disorders involving abortion, stillbirth, and weak fetus [1].

Lifecycle

The life cycle of *Neosporacaninum* is characterized by the presence of three infectious agents, tachyzoites, bradyzoites, and oocytes which are infectious to other animals after puberty in the external environment. Lifecycle completed by the presence of an intermediate and final host. the intermediate host has tachyzoite and bradyzoite intracellular while oocyst finds in the final host .commonly sheep, goat, cow, horse, cat, deer, mouse, rat, an intermediate host while dogs, coyote gray wolves, Australian dingos,

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and Canis lupus dingo, final host.[2] the animal becomes infected by eating contaminated food by eggs or by eating the intermediated host. Fetal resorption, premature birth, mummification, and stillbirth are outputs of the infection of the genital system [3]

It may infect other organs such as the nervous system and cause mild ataxia, tetraparalysis , and neurologic disturbance especially in dogs and equine s [3, 4] the necropsy examination on aborted fetus show non-suppurative encephalitis, hepatitis, and myocarditis

Risk factor

The presence of dogs is the most important risk factor which is the final host of disease and the main cause that is responsible for the spread of the infection[5], bad management of the field and poor sanctification, and low care of diseased animal also regarded risk factor[6,7]. ages of dams also can be considered one of the resins to infected with Neosporosis, dam with > 4 years more susceptible to have the disease than others [8].

Considering the importance of Neospora caninum is not known in sheep in Mosul city in the present study amid to investigate the prevalence of the parasite and how can be affect on hematological and biochemical parameters.

Material and Methods

This study was done from October 2021 to March 2022 on 133 blood serum of sheep that have been taken from many regions of Iraqi Mosul city and the cases were brought to the clinic of the veterinary hospital of collage , Mosul, as well as cases from a specific veterinary clinic.

The blood samples were taken from the jugular vein with a disposable needle 5ml for each sample after cleaning the region with alcohol 70% and let it dry and put 3 ml from blood in gel tube for spread serum by centrifuge 2500rpm for 10 miuntes and keep it in -20^{o}c until use . Serological tests involved iELISA from (ID.Vet, France) and Fast immunochromatographic sandwich technique from (MEGACOE DIAGNOSTIC KIT) tests were done for the samples and each by company instruction in addition to biochemical parameters AST, ALT, CRPby commercial kits (Bio lab) accompanied with Neospora in sheep and (Cholinestrase) manually byelectrometric method (the reaction mixture contained 3 mL distilled water, 0.2 mL Serum and 3 mL of pH 8.1 buffer (barbital-phosphate buffer solution (1.237 g sodium barbital 0.163 g potassium dihydrogen phosphate and 35.07 g sodium chloride/L of distilled water) at 3 mL/100 mg wet weight) . Initial pH of the mixture (pH1) was measured with a glass electrode using a pH meter (Hanna, Romania), and then 0.10 mL of the substrate 7.5% acetylthiocholine iodide was added to the mixture which was incubated at 37 ºC for 30 min. At the end of the incubation period, the pH of the reaction mixture (pH2) was measured. The enzyme activity (expressed as ΔpH/30 min) was calculated as follows: ChE activity (ΔpH/30 min) = (pH1 – pH2) - Δ pH of blank) [9] , and the remaining blood put in EDTA for hematological change such as hematocrit or (PCV) , hemoglobin (HB),Total leucocytes count (TLC), red blood cell count (RBCs) ,mean corpuscular (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) white blood cell count (WBCs) count and differential leucocyte count (DLC) by hematological analyzer for seropositive sample.

Statistical analysis

The study data were statistically analyzed using the programSigmaPlot for windows version 12.0.0., to find standard errors and mean of the result.

Results

Results showedthat 28 out of 133 samples were seropositive to *N.caninum* by iELISA with ratio 21.05 %; and 24 out of 133 seropositive by thefast test with ratio 18.04% as (Table 1).

Hematological analysis showed no alterations in (RBCs count, Hb, PCV) with significant decrease in MCV, MCH in contrast; there is a significant increase in total white blood cell count and DLC, there is also an increase in AST, ALT, ACH and CRP compared with control group (Tables 2,3).

TABLE 1. Percentage of seropositive of sheep infected with *Neosporacanniunm.*

Animal	Number of samples	Rapid test		iELISA	
		Seropositive	Percentage of infection	Seropositive	Percentage of infection
Sheep	133	24	18.04%	28	21.05%

TABLE 2. Hematological parameters of the infected sheep with *Neosporacanniunm.*

Hematological Parameters	Control SE ± mean	Infected sheep SE ± mean
RBC x 10^{12}/l	0.376±8.811	0.714 ± 8.850
Hb g/dl	0.261±8.140	0.190 ±7.854
PCV%	0.965±25.467	0.617 ±26.568
MCV fl	0.686±31.662	27.6721.792±*
MCH pg	0.335±9.375	0.304 ±9.173
MCHC g/dl	0.939±32.104	29.509±0.171*
Total WBC *10^9/l	8.79 ± 0.642	2.31 ±14.05*
Lymphocyte %	4.365 ± 57.963	63.923±5.707*
Neutrophils %	4.390 ± 36.565	38.000±6.419*
Monocyte%	0.347 ± 1.407	1.026 ±3.813*
Eosinophils%	1.333±1.092	1.917±7.889*

TABLE 3. Biochemical parameters of the infected sheep with *Neosporacanniunm.*

Biochemical parameters	Control Mean ± SE	Infected sheep Mean ± SE
AST	52.904 ± 8.025	128.94 ±33.827*
ALT	39.682 ± 5.772	45.39 ± 4.638*
Cholinesterase	0.017± 0.008	0.24± 0.031*

TABLE 4. C-reactive protein concentration of infected sheep with *Neosporacanniunm*.

CRP con. mg/l	12	24	48	96	192	384
N	10	6	5	5	3	0

Discussion

Neosporosis in sheep can be diagnosed by detection of anti *Neosporacanniunm* antibodies using many serological tests. In this study, diagnosis made by two techniques Fast immunochromatographic sandwich technique and iELISA (18.04%, 21.05%, respectively). studies show the disease in Waist, Mosul Iraq [8,10,11] many researches demonstrated the parasite in sheep [12-14] by fast test many studies was show the disturbing of the parasite in countries around world [15-17] due to many risk factors such as presence of dogs , age of the animals, management of the farm . This study was the first serodiagnosis of *Neosporacaninum* by fast immunochromatographic sandwich technique in Mosul city, Iraq.

Some studies showed the distribution of the parasite in other countries around the world [15-17]. Most of these previously studied findings have been nearly similar to our study results and these differences may be explained by the use of the different serological tests in addition to different factors such as sample size ,climatic Factors[18] also this study indicated no significant change in RBCs count, PCV, MCH ,in compare with control group but the decrease was occurred in MCV, MCHC, as a result of anemia due to decrease of appetite occur by the infection , also the result indicated an increase in WBCs such as neutrophils, monocyte ,eosinophil and this is supported by some authors19] ,these high rates are due to the host immune response in defending infected cells by trying to get rid of the parasite specially eosinophil . also , the result shows a significant increase in AST, ALT and cholinesterase.

Neosporacaninum caused a clear alteration in the liver enzymes as a result of the damage to liver cells which linked with climes because of parasitic infection increased in autumn and spring,thus the result agreed with Bottari et al. [20], the result show increase in C-reactive protein, in the presence of antigen all defense cells of animal such as natural killer cell ,B cell, T cell, in addition to acute phase protein involved CRP which stimulated cytokines to remove the damaged tissues and pathogens that indicator for the inflammation this agree with [21] CRP regarded one of protines which named “positive acute phase proteins” which synthesized and production by liver and increased during inflammation [22].

Consent for publication
Not applicable

Competing interests
The authors have no competing interests to declare.

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الانتشار المصلي للنيوسبورا الكلبية وبعض المعايير الدموية والكيموحيوية في في الضأن في مدينة الموصل/ العراق

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استهدفت الدراسة الحالية معرفة نسبة تواجد لضواد داء البوغة الكلبية في امصال الضأن بالاعتماد على الاختبارات المصلية المختلفة والمتمثلة بالاختبار السريع واختبار الممتز المناعي غير المباشر فضلا عن معرفة التغيرات في بعض المعايير الدموية والكيموحيوية المصاحبة للاصابة بداء البوغة الكلبية.

شملت الراسة فحص ١٣٣ عينة دم من مناطق مختلفة من مدينة الموصل ، اظهرت النتائج ان النسبة الكلية لتواجد اضداد داء البوغة الكلبية بأستخدام الاختبار السريع واختبار الممتز المناعي غير المباشر (١٨,٠٤٪ و ٢١,٠٥ ٪) على التوالي.كما بينت النتائج عدم وجود تغير معنوي في عدد كريات الدم الحمر والهيموغلوبين وخلايا الدم المرصوصة و معدل خضاب الدم الكروي مع حدوث انخفاض معنوي بمعدل الحجم الكروي ومعدل تركيز خضاب الدم بالاضافة الى وجود ارتفاع معنوي في عدد الكلي والتفريقي لخلايا الدم البيض مقارنة بمجموعة السيطرة.

لوحظ هناك ارتفاع معنوي في خميرتي الاسبارتيت ناقلة الامين والالنين ناقلة الامين و خميرة الاستايل كولين . مع ظهور ارتفاع في تركيز بروتين C التفاعلي في عينات الامصال الموجبة لأختباري السريع والممتز المناعي غير المباشر في الضأن المصابة بداء البوغة الكلبية مقارنة مع مجموعة السيطرة.