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Serological and biochemical study for *Neospora caninum* in goats/Mosul/Iraq

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Abstract---The aim of the present study was to determine seroprevalence of *Neospora caninum* in goat by using different serological test. Fast test and indirect ELISA and comparative between two test in addition to study of changes in some biochemical parameters accompanied with neosporosis in goat. study included examination of (51) blood serum sample in different areas of Mosul city from October 2021 to March 2022 . Results showed that prevalence of Neosporosis by using Fast test and indirect ELISA was (3.92%,5.88%) respectively Results also showed moderate agreement between two test . Results showed significant increased in Aspartate transaminase, Alanine transaminase, Acetyl cholinesterase and C-reactive protein . all these parameters examined on seropositive sera for Fast test and iELISA in goat infected with Neosporosis compared with control for same test .

Keywords---ELISA, goat, *Neospora*, biochemical study.

Introduction

Neosporosis is one of the recently discovered causes of abortion in the world, it is caused by the parasite *Neospora* , which belongs to the phylum Coccidia, and transmitted by dogs and wolves as its definitive host (Dubey, JP., 2003). The study of researcher (Bjerkas *et al.*, 1984) in Norway indicated that the parasite was isolated and diagnosed for the first time as a cause of encephalitis and myositis in dogs The parasite was observed in different species of birds, but it was not isolated accurately (Dubey *et al.*, 2007; de Barros *et al.*, 2018) The life cycle of the parasite is completed by the presence of the intermediate host and the definitive host. The intermediate host contains the tachyzoite and bradyzoite

stages intracellularly, while the oocyst stage is in the definitive host. Sheep, goats, cattle, horses, cats, wolves, coyotes, deer, and dogs are infected with the parasite. Sheep, goats, cattle, equids, deer, cats, mice, and rats are the common intermediate hosts, while dogs, wolves, and the Coyote gray wolves Australian dingoes (*Canis lupus dingo*) are the definitive host. (Dubey *et al.*, 2002) The disease can be caused by two main ways: the horizontal transmission and the vertical transmission. Neosporosis can infect the reproductive system of animals, especially cows, sheep, goats and deer in particular, causing failure in it and resulting in Fetus adsorption and mummification, as well as abortion, premature birth, or the birth of a tired fetus with abnormalities in the fetus. (Wouda W, 2000)

The study of the researcher (Maganga *et al.*, 2016) in the countries of Gabon and Pibora indicated that the possibility of sheep being infected with the disease increases by four times due to the presence of street dogs significantly more in these countries than in the rest of the countries and this is a result of poor handling of waste in rural communities and poor sterilization resulting in garbage dumps, which be a good food source for these dogs (Maganga *et al.*, 2016). In diagnosing the disease, researchers relied on many techniques, including immunosorbent test and indirect immunofluorescence technique from samples of blood serum, blood plasma and milk (Shaapan., 2016).

Because of the importance of the disease and its close relationship to the economic situation of livestock because of the losses it causes, this study was conducted, which aimed to:

- 1- Diagnosis of canine neosporosis using serological methods represented by using the indirect immunosorbent test and the rapid test in goats.
- 2- Comparing the efficiency of the aforementioned two methods.
- 3- Recording some biochemical changes.

Materials and Methods

The study was conducted on 61 serum samples (10 of which were used as a control) distributed over different areas of Mosul city and for the period from October 2021 to March 2022. These samples included aborted animals and healthy animals. Blood was collected from the jugular vein using disposable syringes with a capacity of 3 ml after sterilizing the area with 70% absolute ethyl alcohol and leaving it, and the blood was placed in plastic tubes that do not contain anticoagulant (containing gel) to separate the blood serum using a centrifuge at a speed of 2500 rpm, for a period of 10 minutes after that, the serum was kept at -20°C until the serological and biochemical tests were performed. The other part of the blood was placed in tubes containing an anticoagulant for blood tests(EDTA). Serological tests included indirect ELISA for the detection of antibodies against *N.caninum* supplied by (ID.vet, France) and Rapid immunochromatographic sandwich technique according to the manufacturer's instructions (MEGACOE DIAGNOSTICKIT). Whereas, the biochemical tests were represented by measuring the activity of hepatic enzymes (aspartate transaminase and alanine transaminase), measuring the activity of acetylcholine and measuring the concentration of C-reactive protein. The activity

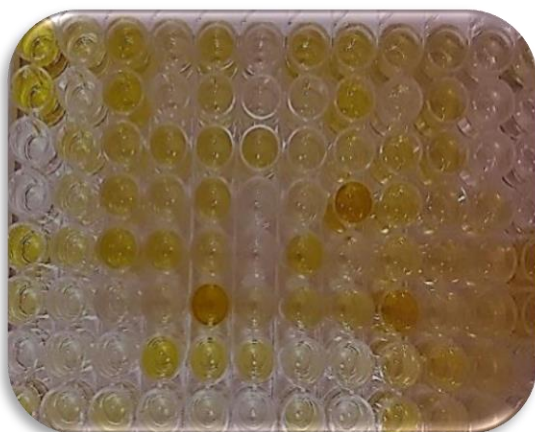
of hepatic enzymes and C-reactive protein was measured based on ready-made standard solutions according to the instructions of the supplied company, as for the measurement of acetylcholine, it was relied on the electrometric method based on (Mohammad et al.,1997). The results were statistically analyzed using SPSS software.

Results

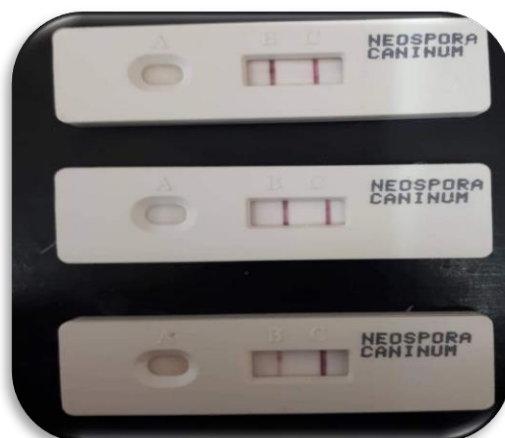
The results showed that by examining 51 blood serum samples for goats, the total percentage of antibodies to canine neosporosis using the rapid and indirect immunosorbent assays, amounted to 3.92, 5.88% respectively, as shown in Table (1).

Table (1) Presence of IgG antibodies in goats according to the rapid and indirect immunosorbent assays

Animal	No. of examined samples	Rapid test		ELISA	
		Positive numbers	Percentage%	Positive numbers	Percentage%
Goat	51	2	3.92	3	5.88



Picture No. (1) represents the positive result of ELISA



Picture No. (2) shows the positive result of the rapid test

The results of the study also showed a moderate agreement between the rapid immunochromatographic sandwich technique test and the ELISA test in diagnosing of canine neosporosis infection in the animals of the study based on the Kappa value which amounted (0.440). While the results of biochemical tests on the blood serum samples of goats, which gave positive results for the indirect immunosorbent test, showed a significant increase ($P < 0.05$) in aspartate transaminase and alanine transaminase, as well as a significant increase in the activity of cholinesterase compared with the control group, as shown in Table (2).

Table (2) Changes in some biochemical parameters in goat serum associated with the presence of IgG antibodies compared to the control group

Biochemical parameters	Infected Goats Mean± SE	Control Mean ± SE
Aspartate transaminase IU/liter	*102.714± 12.528	98.813±12.099
Alanine transaminase IU/liter	*50.286±11.281	42.190±5.172
Cholinesterase activity	*0.219±0.021	0.0567±0.0145

* Values are significantly different under the probability level ($P < 0.05$).

When studying the C-reactive protein for the positive samples, the concentration of the C-reactive protein ranged between (12-96) mg/L and it was distributed according to the pattern shown in Table No. (3)

Table (3) shows the numbers of cases for C-reactive protein concentrations

CRP concentration mg/l	12	24	48	96	192	384
No. of cases	1	2	1	1	0	0

Discussion

Studies have proven the importance and severity of the disease due to the economic losses it causes and the possibility of diagnosing the disease in several ways. The method of culture in tissue cultures is one of the important ways to investigate the parasite. Due to the difficulty of this method and its need for a long time, other techniques represented by serological techniques have been relied upon, such as the indirect immunosorbent technology and the rapid technique, each of which depends on the presence of IgG antibodies in the sera of infected animals. (Maganga *et al.*, 2016) The results of the current study showed that the infection rate was 3.92% and 5.88% in both the rapid test and the indirect immunosorbent test, respectively, and thus the results of the study are close to the study of the researcher (Sharma *et al.*, 2015) in his study on goats in southern India using the indirect immunosorbent test.

The study also agreed with the findings of the researcher (Braz *et al.*, 2018) on goat herds from different farms in Brazil, but it differed with (Ghattof and Faraj *et al.*, 2015), the variation in the positive percentages of infected animals is due to the weather and the method of breeding in the fields, taking into account the number of animals in one herd, the density and the size of the sample taken for examination, taking into account the method used in diagnosing the disease. Biochemical tests in goats showed a significant increase in the value of aspartate transaminase (AST) and alanine transaminase (ALT), the rise of these enzymes is due to infection with the parasite, as the canine neosporosis parasite is like other parasites, which causes a clear change in liver enzymes as a result of damage to the liver cells and abnormality of the cell unite due to pathological lesion made by it. Thus, the study agreed with the researcher (Bottaria *et al.*, 2014).

The activity of the cholinesterase ACH was measured and it was found to be high in seropositive in compare with control group . It should be noted that cholinesterase is a neurotransmitter that rises in the inflammatory response that is synthesized from choline and acetyl co enzyme in the cell cytoplasm before presynaptic terminal (Hill et al., 2004). The high activity of cholinesterase is attributed to the inflammatory response caused by the parasite and to its pathogenesis, as it stimulates the cholinergic system to secrete more defenses to resist the parasite. This result agreed with the researcher (Tonina *et al.*, 2015; Appelt *et al.*, 2019), where they observed an increase in cholinesterase levels in cases of canine neosporosis infection.

The C-reactive protein present in seropositive samples was measured and it was found that they were suffering from an increase in the value of this protein, as the protein concentration in the sample ranged between (12-192) mg/l with a difference in the number of samples. C-reactive protein consider one of acute phase proteins involve haptoglobin, fibrinogen, ceruloplasmin, seromucoid, C-reactive protine (Vidhi et al., 2011), which stimulated by pathogens as a result of cell injury, it activated during the first 24-48 hours from infection and continue to the recovery (Ballou et al., 1992) The results of the study agreed with what was mentioned by the researcher (Appelta *et al.*, 2019), where the examination of C-reactive protein is an indicator of inflammation in the body, as the level of the protein increases as a result of stimulating the immune system. the acute phase protine regarded one of the mechanisms which that take place to get rid of and replace of damaged tissue (Vidhi et al., 2011), C-Reactive protine has very important rule in activation of compiement and in opsonisation and production of cytokinase, macrophage and monocyte. (Ballou and, Lozanski;1992)

Conclusion

This report showed a widespread of *Nespora caninum* in Mosul city however needed more researches in future . there is a moderate agreement between iELISA and fast test have been used . seroprevalance sample show change of AST,ALT,ACH and CRP concentration .

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