Research Article

Biochemical Effects of *Toxoplasma gondii* and *Neospora caninium* Infection on Diary Bovine Models in Menoufia Province, Egypt

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Abstract | The current study was carried out to illustrate the biochemical effects of *Toxoplasma gondii* and *Neospora caninium* infection in local water buffaloes and cows from Menoufia Province, Egypt. One hundred and ten serum samples collected from *Toxoplasma* or *Neospora* naturally-infected or non-infected water buffaloes and cows were used to study the effects of toxoplasmosis and neosporosis on bovine animals' health. Serum samples were classified into thirteen groups according to acute and chronic infections. Toxoplasmosis and neosporosis showed significant changes in the liver function and lipid profile parameters during both acute and chronic *T. gondii* and *N. caninum* infections compared to control group. Also, our results showed a significant increase of the inflammatory marker C-reactive protein in all examined infected groups compared to the control group. While there were no significant differences on the level of albumin, globulin, total protein, and some kidney functions as compared to the control group. In conclusion, both toxoplasmosis and neosporosis had unpropitious effects on the bovine animal health.

Keywords | *Toxoplasma gondii, Neospora caninum*, Liver functions, Bovine, Lipid profile

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INTRODUCTION

The closely related protozoan parasites, *Toxoplasma gondii* and *Neospora caninum* have an indirect life cycle with carnivores as the definitive hosts, distributed worldwide and can infect a wide range of animal species. In the host, *T. gondii* and *N. caninum* can cause abortion or neonatal mortalities (Anderson et al., 1991; Dubey, 2003; Guo et al., 2015). While toxoplasmic encephalitis is a life-threatening central nervous system infection observed in the later stages of HIV infection (Luft and Remington, 1992), *N. caninum* result in neurologic alterations in dogs (Barber and Trees, 1996). As a result of either acute infection or reactivation of infection *T. gondii* infection can be life threatening for congenitally infected infants, immunocompromised, and immunodeficient patients (Frenkel and Escajadillo, 1987; Chintana et al., 1998; Luft and Remington, 1988; Aspinall et al., 2003). In animals, both parasites infection result in significant reproductive losses, and hence economic losses, (Faria et al., 2007; Donahoe et al., 2015).

In previous surveys from Egypt, *T. gondii* and *N. caninum* antibodies were demonstrated in the sera from asymptomatic pregnant women (Ibrahim et al., 2009a; El-Shqanqery et al., 2017; Ibrahim et al., 2017; chickens (El-Massey et al., 2000; Ibrahim, 2013, 2016) ducks, pigeons, sheep (Dubey et al., 2003; Ibrahim, 2016; Ibrahim et al., 2017, 2018), camels, and water buffalo (Hilali et al., 1998; Dubey et al., 1998).

In cattle and water buffalos, *N. caninum* can affect animal productivity (Chryssafidis et al., 2011), resulting in reproductive losses such as abortions and neonatal mortality (Dubey et al., 2007; Chryssafidis et al., 2015). Furthermore, viable *T. gondii* was isolated from naturally

infected aborted bovine fetuses (Canada et al., 2002). Therefore, both diseases are considered important economic diseases in animals, and zoonosis of *Toxoplasma* results in a variety of clinical manifestations in humans.

In the Egyptian markets, cattle and water buffaloes are representing the main sources of red meat (Alboghdady and Alashry, 2010). Several previous studies reported the unpropitious consequences of *Toxoplasma* and *Neospora* on the host performance and health status (Mahboub et al., 2013; Donahoe et al., 2015; El-Sayed et al., 2016; Didiano et al., 2020). In the current study, our objective was to estimate the biochemical effects of *Toxoplasma gondii* and *Neospora caninium* on naturally infected cows and water buffalos in Menoufia Province, Egypt.

MATERIALS AND METHODS

Ethics statement

This study was performed after getting permission from the Institutional Animal Ethical Committee, Menoufia University, Egypt (approval ID: MUFS/F/IM/2/17).

Blood samples

Out of five hundred examined bovine samples, one hundred and ten blood samples, normal, healthy and naturally infected with *Toxoplasma* and/or *Neospora*, were collected from water buffalo and cow (63 water buffalo samples, 47 cow samples). The samples were incubated at room temperature for one hour, centrifuged at 3000 rpm for 15 minutes and then sera were collected and stored at -20 °C until analysis.

Experimental design

Collected serum samples were divided into 13 groups (7 groups of water buffalo's samples and 6 groups of cow's samples) according to the status of infection. The infection status was confirmed by highly specific *Tg*SAG2 and *Nc*SAG1 based ELISA (Ibrahim et al., 2009a).

Water buffalo's serum samples

Group 1: Normal, healthy serum samples (n=10); Group 2: Chronically infected serum samples with *T. gondii* (positive for IgG antibodies) (n=9); Group 3: Acutely infected serum samples with *T. gondii* (positive for IgM antibodies) (n=11); Group 4: Chronically infected serum samples with *N. caninum* (positive for IgG antibodies) (n=11); Group 5: Acutely infected serum samples with *N. caninum* (positive for IgM antibodies) (n=10); Group 6: Chronically infected serum samples with *T. gondii* and *N. caninum* mixed infection (n=8); Group 7: Acutely infected serum samples with *T. gondii* and *N. caninum* mixed infection (n=4).

Cow's serum samples

Group1: Normal, healthy serum samples (n=10); Group 2: Chronically infected serum samples with *T. gondii* (positive for IgG antibodies) (n=7); Group 3: Acutely infected serum samples with *T. gondii* (positive for IgM antibodies) (n=7); Group 4: Chronically infected serum samples with *N. caninum* (positive for IgG antibodies) (n=10); Group 5: Acutely infected serum samples with *N. caninum* (positive for IgM antibodies) (n=10); Group 6: Chronically infected serum samples with *T. gondii* and *N. caninum* mixed infection (n=3). In the current study no mixed *T. gondii* and *N. caninum* acute infection was detected in cow's samples.

Biochemical analysis

Aspartate transaminase (AST) activity, alanine transaminase (ALT) activity, and alkaline phosphatase (ALP), as they produced intracellular and their elevated serum levels indicate hepatocytes damage, were measured using kinetic kits (Human Diagnostic Kits, Germany). Also total protein, albumin, total bilirubin, and direct bilirubin were detected using colorimetric method kits (Diamond Diagnostics kit, Egypt). Blood urea and serum creatinine, indicate kidney damage, were examined using (Diamond Diagnostic kit, Egypt). Uric acid was measured using Spinreact diagnostics kit (Spain). Lipid profile (cholesterol, triglycerides, and high density lipoprotein [HDL]) was measured using Spinreact diagnostic kits (Spain). Low density lipoprotein (LDL) was measured indirectly using the Friedewald formula which incorporates total cholesterol, HDL and triglyceride concentrations: LDL (mg/dl) = total cholesterol (mg/dL) – HDL (mg/dL) – triglyceride (mg/dL)/5 (Friedewald et al., 1972; Tremblay et al., 2004). The serum globulin level was calculated by subtracting the obtained serum albumin concentration from the obtained serum total protein concentration (Ibrahim et al., 2019). Finally, the inflammatory marker C-reactive protein (CRP) was detected using Biosystems diagnostics kits (Spain).

Statistical analysis

Using SPSS, data were subjected to statistical significance tests using one-way analysis of variance (ANOVA), followed by post hoc analysis of group differences that was accomplished by the least significant differences (LSD) test; $P<0.05$ was considered to be statistically significant.

RESULTS

The results of water buffalos in the present study showed that there was a significant increase in serum AST activity in samples with chronic infection of *T. gondii* while there is no significant change during chronic infection of *N. caninum* or mixed chronic infection of *N. caninum* and *T. gondii*. On the level of ALP activity, a significant increase was detected in all chronically infected groups compared

with the control group. There is also a significant increase in cholesterol, triglyceride and LDL in all chronically infected groups as compared with the control group. Chronic mixed infection resulted in an evaluation of HDL levels without any significant change when compared to the control healthy group. Finally, there is a significant increase in CRP compared with the control group in the chronically infected water buffalo's samples. No significant changes were detected in all other examined parameters in comparison with the control group (Table 1).

Table 2 revealed that there is a significant increase in serum ALP activity in all acutely infected groups when compared to the control group. Moreover, a significant increase was detected on the activity of AST in the acutely infected *N. caninum* group as compared to control group. The activity of this enzyme was elevated in the acutely infected *T. gondii* group and acutely mixed infection without any significant changes. Urea, as a kidney function parameter, showed a significant increase in the *Toxoplasma* infected group during the acute infection in water buffalo's samples. Cholesterol and LDL level were significantly elevated in the acutely *N. caninum* infected group when compared to the control group. Higher level of cholesterol and LDL was detected in *T. gondii* and mixed infection during the acute infection without any significant differences. Triglycerides and CRP showed a significant increase in all acutely infected groups in comparison with the control healthy group. No significant changes were detected in all other examined parameters in comparison with the control group.

Table 1: Biochemical analysis of chronically infected water buffalos with toxoplasmosis and/or neosporosis.

Parameters	Control	*T. gondii* +ve IgG	*N. caninum* +ve IgG	Mixed infection +ve IgG
AST (U/L)	102.75±4.05	129.68±7.52*	112.22±4.14	95.80±6.29
ALT (U/L)	57.90±2.30	59.52±2.66	59.52±2.66	51.94±3.12
ALP (U/L)	80.62±8.06	149.60±17.80*	133.20±6.59*	128.00±4.43*
Albumin (g/dl)	4.08±0.07	3.98±0.06	3.89±0.07	3.85±0.11
Total protein (g/dl)	6.75±0.19	6.89±0.13	7.04±0.10	6.58±0.21
Globulin (g/dl)	2.71±0.17	2.91±0.15	3.15±0.12	2.73±0.16
Total bilirubin (mg/dl)	0.52±0.02	0.53±0.03	0.53±0.03	0.59±0.04
Direct bilirubin (mg/dl)	0.11±0.01	0.13±0.01	0.20±0.05	0.13±0.02
Urea (mg/dl)	45.65±0.90	49.16±1.56	48.76±0.78	49.11±1.81
Creatinine (mg/dl)	1.24±0.02	1.27±0.02	1.28±0.02	1.20±0.07
Uric acid (mg/dl)	3.74±0.15	3.75±0.10	3.69±0.13	3.32±0.23
Cholesterol (mg/dl)	96.82±9.32	149.75±14.68*	157.45±10.34*	168.69±12.85*
Triglyceride (mg/dl)	54.17±9.49	155.75±33.35*	98.16±14.68*	125.14±25.40*
HDL (mg/dl)	36.29±2.20	38.96±2.04	36.63±1.46	43.60±1.95
LDL (mg/dl)	61.00±8.06	84.51±8.88*	99.89±7.89*	111.12±9.37*
CRP (mg/l)	2.88±0.97	11.14±2.4*	12.75±2.64*	14.40±4.07*

Data are expressed as: Means ± SE. * Indicates significant difference ($P < 0.05$).

Table 2: Biochemical analysis of acutely infected water buffalos with toxoplasmosis and/or neosporosis.

Parameters	Control	*T. gondii* +ve IgM	*N. caninum* +ve IgM	Mixed infection +ve IgM
AST (U/L)	102.75±4.05	115.55±5.99	124.56±7.04*	112.85±10.11
ALT (U/L)	57.90±2.30	60.18±3.63	61.54±4.12	56.40±2.85
ALP (U/L)	80.62±8.06	130.78±8.83*	132.49±11.06*	111.52±5.97*
Albumin (g/dl)	4.06±0.08	3.93±0.07	3.89±0.08	3.95±0.09
Total protein (g/dl)	6.77±0.19	6.91±0.09	6.66±0.21	6.37±0.32
Globulin (g/dl)	2.70±0.17	2.98±0.11	2.77±0.20	2.42±0.25
Total bilirubin (mg/dl)	0.52±0.02	0.58±0.03	0.57±0.03	0.58±0.05
Direct bilirubin (mg/dl)	0.11±0.01	0.15±0.01	0.15±0.02	0.13±0.03
Urea (mg/dl)	45.65±0.90	51.51±1.33*	49.24±1.55	44.13±2.25
Creatinine (mg/dl)	1.24±0.02	1.27±0.02	1.22±0.02	1.11±0.12
Uric acid (mg/dl)	3.74±0.15	3.76±0.13	3.81±0.11	3.28±0.26
Cholesterol (mg/dl)	96.82±9.32	137.93±12.23	161.22±17.95*	144.05±29.94
Triglyceride (mg/dl)	54.17±9.49	112.61±17.61*	107.09±11.35*	91.83±21.09*
HDL (mg/dl)	36.29±2.20	38.57±1.35	43.03±1.26	39.58±2.25
LDL (mg/dl)	61.00±8.06	85.18±8.63	100.50±16.48*	86.11±26.95
CRP (mg/l)	2.49±0.61	12.67±2.33*	12.75±2.64*	8.00±2.00*

Data are expressed as: Means ± SE; * Indicates significant difference ($P < 0.05$).

The results of cows in the present study illustrated that there is a significant increase in serum AST, ALT activity only in mixed chronically infected samples with no significant increase in other chronically infected groups as compared to the control group. ALP activity was increased significantly in *T. gondii* and *N. caninum* chronically infected groups compared with the control group, while the activity of this enzyme was elevated in the mixed chronically infected group without any significant change. Total bilirubin showed a significant increase in the chronically *N. caninum* infected group when compared to control group. Urea and creatinine levels were elevated in the mixed chronically infected group without any significant changes when compared to the control group. Uric acid showed a significant increase in chronically *T. gondii* infected group. Cholesterol and triglyceride showed a significant increase in chronically *N. caninum* infected group, while there is an elevation in those parameters in chronically *T. gondii* infected group and mixed chronically infected group without any significant changes. Finally, LDL and CRP showed a significant increase in all chronically infected groups when compared with control group (Table 3).

Table 3: Biochemical analysis of chronically infected cows with toxoplasmosis and/or neosporosis

Parameters	Control	*T. gondii* +ve IgG	*N. caninum* +ve IgG	Mixed infection +ve IgG
AST (U/L)	56.31±2.14	48.72±2.62	49.51±3.44	111.93±16.00*
ALT (U/L)	29.61±1.96	29.07±2.47	27.89±1.93	43.37±8.36*
ALP (U/L)	109.90±18.54	161.17±18.51*	193.89±27.64*	136.63±38.72
Albumin (g/dl)	3.92±0.09	3.80±0.16	3.74±0.15	3.96±0.08
Total protein (g/dl)	7.09±0.27	7.69±0.20	7.50±0.22	6.94±0.37
Globulin (g/dl)	3.17±0.23	3.89±0.31	3.75±0.16	2.98±0.44
Total bilirubin (mg/dl)	0.63±0.03	0.63±0.05	0.80±0.04*	0.51±0.02
Direct bilirubin (mg/dl)	0.16±0.01	0.18±0.02	0.21±0.02	0.11±0.02
Urea (mg/dl)	34.90±1.54	40.10±0.58	39.25±1.56	43.97±5.69
Creatinine (mg/dl)	0.83±0.06	0.86±0.10	0.89±0.06	1.18±0.06
Uric acid (mg/dl)	3.84±0.10	4.39±0.23*	4.11±0.13	3.53±0.33
Cholesterol (mg/dl)	119.91±13.04	159.03±26.27	206.62±22.85*	145.50±9.85
Triglyceride (mg/dl)	56.09±7.35	63.01±16.42	166.00±26.19*	77.13±18.97
HDL (mg/dl)	37.81±1.57	39.03±0.75	39.53±1.40	38.10±2.99
LDL (mg/dl)	59.68±6.67	125.40±22.45*	159.76±18.93*	91.97±10.44*
CRP (mg/l)	3.08±1.1	10.00±2.97*	15.00±2.78*	12.00±0.00*

Data are expressed as: Means ± SE. * Indicates significant difference ($P < 0.05$).

Table 4: Biochemical analysis of acutely infected cows with toxoplasmosis or neosporosis.

Parameters	Control	*T. gondii* +ve IgM	*N. caninum* +ve IgM
AST (U/L)	56.31±2.14	50.29±4.78	51.69±3.15
ALT (U/L)	29.61±1.96	28.80±1.90	32.11±2.59
ALP (U/L)	109.90±18.54	140.20±21.58	210.38±30.42*
Albumin (g/dl)	3.92±0.90	4.02±0.18	3.87±0.10
Total protein (g/dl)	7.09±0.27	7.63±0.20	7.55±0.20
Globulin (g/dl)	3.17±0.23	3.61±0.20	3.69±0.16
Total bilirubin (mg/dl)	0.63±0.03	0.67±0.05	0.72±0.04
Direct bilirubin (mg/dl)	0.16±0.01	0.17±0.01	0.16±0.01
Urea (mg/dl)	33.48±1.96	46.34±3.00*	39.36±2.44
Creatinine (mg/dl)	0.83±0.06	0.89±0.08	0.75±0.04
Uric acid (mg/dl)	3.84±0.10	3.84±0.09	3.75±0.11
Cholesterol (mg/dl)	119.91±13.04	185.43±25.37	139.60±13.94
Triglyceride (mg/dl)	50.96±2.42	49.96±10.04	46.28±3.27
HDL (mg/dl)	37.81±1.57	41.74±1.53	39.49±1.52
LDL (mg/dl)	59.68±6.67	141.44±22.97*	94.83±14.10*
CRP (mg/l)	2.91±0.97	14.40±4.07*	11.14±2.42*

Data are expressed as: Means ± SE. * Indicates significant difference ($P < 0.05$).

Table 4 showed that there is a significant increase in serum ALP activity in the acute *N. caninum* infected group as compared with the control group. There is an elevation in ALP level in acutely *T. gondii* infected group without any significant change. Moreover, urea was significantly increased in acutely *T. gondii* infected group, while there was an elevation in the acutely *N. caninum* infected group without any significant change as compared with the control group. Higher level of cholesterol was detected in *T. gondii* and *N. caninum* during the acute infection without any significant difference compared with the control group. Finally, LDL and CRP showed a significant increase in all acutely infected groups compared with the control group. No significant changes were detected in all other examined parameters in comparison with the control group.

DISCUSSION

In the present study, we investigated the effects of *T. gondii* and *N. caninum* infection on water buffalos and cow's health through examining the changes in some biochemical parameters of the infected animals in comparison with normal healthy animals. These parameters give an indication about the effects of these causative agents on animals' health and performance.

In the current work, toxoplasmosis and neosporosis increase the activity of serum liver enzymes. In the examined bovine samples, ALP activity was elevated obviously in *T. gondii*, *N. caninum* and mixed chronically and acutely infected groups compared with the control group. Moreover, there is a significant increase in serum AST, ALT activity in mixed chronically infected cow samples, and a significant elevation in AST activity in samples with chronic infection of *T. gondii* and acute infection of *N. caninum* in water buffaloes. These findings were in line with that indicated by (Suzuki., 1971; Portugal et al., 2004; Al-Kaysi et al., 2010; Al-Hussary and Al-Zuhairy, 2010; Alekish et al., 2017; Didiano et al., 2020), who found that the activities of AST and ALT were increased in *T. gondii* and *N. caninum* infected animals. In humans, the results of *Toxoplasma* positive patients showed a significant elevation in the liver enzymes: AST, ALT, and ALP in the sera compared with negative patients (Al-Khamesi et al., 2016; El-Sayed et al., 2016). These changes may be due to the liver damage caused by *Toxoplasma* and/or *Neospora* infection, which increased the release of the liver enzymes in the serum. Previous studies recorded that *Toxoplasma* or *Neospora* infection resulted in extensive damage in the liver cells (Blais et al., 1993; Calderaro et al., 2009; Donahoe et al., 2015; El-Sayed et al., 2016; Alekish et al., 2017).

In the current study, urea level was elevated obviously in *N. caninum* acutely infected bovine samples. Furthermore, urea, uric acid and creartinine levels were increased in *T. gondii* and the mixed chronically infected bovine samples as compared with control samples. The elevation in the urea concentration may due to *Toxoplasma* deleterious effects on the kidney which decrease the excretion of urea from the body and subsequently increased its serum level (Mahboub et al., 2013). *Toxoplasma* and *Neospora* were found in the kidneys of the infected animals and led to pathological changes in their tissues (Fayed et al., 2004; Hammouda et al., 2006; Gharadaghi et al., 2012; Donahoe et al., 2015).

The present study revealed that *Toxoplasma* and *Neospora* infection increased serum cholesterol, triglyceride, and LDL levels in chronically infected animals and cholesterol and LDL levels in acutely infected animals. Our finding was in agreement with previous studies (Stockham and Scott, 2002; Mahboub et al., 2013; Didiano et al., 2020). Also previous reports found that parasitic and HIV infection elevates lipoproteins like HDL, LDL and total cholesterol levels (Djoumessi, 1989; Rimland et al., 2006). *Toxoplasma* uses the host metabolic products for its own metabolic pathways (Al-Kennany, 2007). It is well known that cholesterol is synthesized in the endoplasmic reticulum through the main enzyme of the mevalonate pathway, hydroxymethylglutaryl-CoA reductase and further utilized by the cell for synthesis of cholesterol derivatives or membrane biogenesis. Previous microarray analysis using *T. gondii*-infected human foreskin fibroblasts showed up-regulation of key genes involved in the mevalonate pathway and cholesterol synthesis (Blader et al., 2001). This included an increase in expression level of hydroxymethylglutaryl-CoA reductase (Blader et al., 2001). Nishikawa et al. (2011) detected that in macrophages the growth of intracellular *T. gondii* was contributed to the host cholesterol synthesis.

Our study demonstrated that *Toxoplasma* and/or *Neospora* infection increased the inflammatory marker CRP levels in acutely and chronically infected animals when compared to the control animals. This elevation may due to the inflammation related effect of these pathogens in different tissues of the infected animals. Several previous studies recorded the abilities of these parasites and their related proteins to enhance the production of many pro-inflammatory mediators such as interleukin-6, interleukin-8, tumor necrosis factor alpha and nitric oxide during their infection (Ibrahim et al., 2009b; Ibrahim and Nishikawa, 2016; Jimenez-Pelayo et al., 2019).

CONCLUSION

Both toxoplasmosis and neosporosis negatively affected the liver function, lipid profile and inflammatory status, which reflect harmful consequences on dairy bovine animals' health and performance. Further studies will be needed to define the mechanisms of these consequences.

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AUTHOR'S CONTRIBUTIONS

HMI designed the study. NMB, HMI and AMA performed most of the experiments, analyzed and interpreted the data. NMB and HMI wrote the first version of the manuscript. HMI, AAHA and NMB assisted during the analysis and interpretation of data and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

- Alboghdady MA, Alashry MK (2010). The demand for meat in Egypt: an almost ideal estimation. Afr. J. Agric. Resour. Econ., 4(1): 70-81.
- Alekish MO, Talafha AQ, Alshehabat MA, Bani-Ismail ZA (2017). Relationships between certain metabolic diseases and selected serum biochemical parameters in seropositive dairy cows against *Neospora caninum* infection in different stages of lactation. Annal. Parasitol., 63(2): 99–103.
- Al-Hussary NAJ, Al-Zuhairy ASM (2010). Effect of toxoplasmosis and brucellosis on some biochemical parameters in ewes. Iraq J. Vet. Sci., 24: 73-80. https://doi.org/10.33899/ijvs.2010.5609
- Al-Kaysi AM, Eid RAA, Fahmy BGA (2010). Biochemical studies on the effect of *Toxoplasma* infection on liver and kidney functions in mice. Egypt. J. Comp. Path. Clinic. Path., 23: 174-185.
- Al-Kennany ER (2007). Pathological study on the capability of *Toxoplasma gondii* to induce oxidative stress and initiation a primary lesion of atherosclerosis experimentally in broiler chickens. J. Anim. Vet. Adv., 6: 938-942.
- Al-Khamesi MB, Al-Sibahi ZN, Al-Obaidy LH, Hilal ZH (2016). Studying of kidney, liver functions and some blood ions. In: Toxoplasmosis patients. Al- Mustansiriyah J. Sci., 27(1): 43-46.
- Anderson ML, Blanchard PC, Barr BC, Dubey JP, Hoffman RL, Conrad PA (1991). *Neospora*-like protozoan infection as a major cause of abortion in California dairy cattle. J. Am. Vet. Med. Assoc., 198: 241–244.
- Aspinall TV, Guy EC, Roberts KE, Joynson DH, Hyde JE, Sims PF (2003). Molecular evidence for multiple *Toxoplasma gondii* infections in individual patients in England and Wales: Public health implications. Int. J. Parasitol., 33: 97–103. https://doi.org/10.1016/S0020-7519(02)00230-8
- Barber JS, Trees AJ (1996). Clinical aspects of 27 cases of Neosporosis in dogs. Vet. Rec., 139: 439–443. https://doi.org/10.1136/vr.139.18.439
- Blader IJ, Manger ID, Boothroyd JC (2001). Microarray analysis reveals previously unknown changes in *Toxoplasma gondii*-infected human cells. J. Biol. Chem., 276: 24223–24231. https://doi.org/10.1074/jbc.M100951200
- Blais J, Garneau V, Chamberland S (1993). Inhibition of *Toxoplasma gondii* protein synthesis by azithromycin. Antimicrob. Agents Chemother., 37(8): 1701-1703. https://doi.org/10.1128/AAC.37.8.1701
- Calderaro S, Peruzzi S, Piccolo G, Montecchioni S, Rossi S, Chezz C, Eeltori G (2009). Laboratory diagnosis of *Toxoplasma gondii* infection. Int. J. Med. Sci., 6: 135-136. https://doi.org/10.7150/ijms.6.135
- Canada N, Meireles CS, Rocha A, da Costa JM, Erickson MW, Dubey JP (2002). Isolation of viable *Toxoplasma gondii* from naturally infected aborted bovine fetuses. J. Parasitol., 88: 1247–1248. https://doi.org/10.1645/0022-3395(2002)088[1247:IOVTGF]2.0.CO;2
- Chintana T, Sukthana Y, Bunyakai B, Lekkla A (1998). *Toxoplasma gondii* antibody in pregnant women with and without HIV infection. Southeast Asian J. Trop. Med. Publ. Health, 29(2): 383-386.
- Chryssafidis AL, Cantón G, Chianini F, Innes EA, Madureira EH, Soares RM, Gennari SM (2015). Abortion and foetal lesions induced by *Neospora caninum* in experimentally infected water buffalos (*Bubalus bubalis*). Parasitol. Res., 114(1): 193-199. https://doi.org/10.1007/s00436-014-4178-0
- Chryssafidis AL, Soares RM, Rodrigues AA, Carvalho NA, Gennari SM (2011). Evidence of congenital transmission of *Neospora caninum* in naturally infected water buffalo (*Bubalus bubalis*) fetus from Brazil. Parasitol. Res., 108(3): 741-743. https://doi.org/10.1007/s00436-010-2214-2
- Didiano A, Monti P, Taeymans O, Cherubini GB (2020). Canine central nervous system neosporosis: clinical, laboratory and diagnostic imaging findings in six dogs. Vet. Rec. Case Rep., 8: e000905. https://doi.org/10.1136/vetreccr-2019-000905
- Djoumessi S (1989). Serum lipids and lipoproteins during malaria infection. Pathol. Biol., 37(8): 909-911.
- Donahoe SL, Lindsay SA, Krockenberger M, Phalen D, Šlapeta J (2015). A review of neosporosis and pathologic findings of *Neospora caninum* infection in wildlife. Int. J. Parasitol. Parasites Wildl., 4(2): 216-238. https://doi.org/10.1016/j.ijppaw.2015.04.002
- Dubey JP (2003). Review of *Neospora caninum* and Neosporosis in animals. Korean J. Parasitol., 41: 1-16. https://doi.org/10.3347/kjp.2003.41.1.1
- Dubey JP, Graham DH, Dahl E, Hilali M, El-Ghaysh A, Sreekumar C, Kwok OC, Shen SK, Lehmann T (2003). Isolation and molecular characterization of *Toxoplasma gondii* from chickens and ducks from Egypt. Vet. Parasitol., 30: 89–95. https://doi.org/10.1016/S0304-4017(03)00133-X
- Dubey JP, Romand S, Hilali M, Kwok OC, Thulliez P (1998). Seroprevalence of antibodies to *Neospora caninum* and *Toxoplasma gondii* in water buffaloes (*Bubalus bubalis*) from Egypt. Int. J. Parasitol., 3: 527–529. https://doi.org/10.1016/S0020-7519(97)00190-2
- Dubey JP, Schares G, Ortega-Mora LM (2007). Epidemiology and control of Neosporosis and *Neospora caninum*. Clin. Microbiol. Rev., 20(2): 323–367. https://doi.org/10.1128/CMR.00031-06
- El-Massey A, Mahdy OA, El-Ghaysh A, Dubey JP (2000). Prevalence of *Toxoplasma gondii* antibodies from sera of turkeys, chickens, and ducks from Egypt. J. Parasitol., 32: 99–105. https://doi.org/10.2307/3284886

- El-Sayed NM, Ramadan ME, Ramadan ME (2016). *Toxoplasma gondii* infection and chronic liver diseases: evidence of an association. Trop. Med. Infect. Dis., 1(1): 7. https://doi.org/10.3390/tropicalmed1010007
- El-shqanqery HE, Ibrahim HM, Mohamed AH, El-sharawy AA (2017). Seroprevalence of *Toxoplasma gondii* infection and associated risk factors among asymptomatic pregnant females in Egypt. J. Egypt. Soc. Parasitol., 47(1): 93-100.
- Faria EB, Gennari SM, Pena HF, Athayde AC, Silva ML, Azevedo SS (2007). Prevalence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in goats slaughtered in the public slaughterhouse of Patos city, Paraíba State, Northeast region of Brazil. Vet. Parasitol., 149: 126–129. https://doi.org/10.1016/j.vetpar.2007.07.009
- Fayed HM, Allam KA, Ali NE (2004). Merogony of *Toxoplasma gondii* (Apicomplexa: Coccidia) and its effect on the mortality and histopathology in the house mouse *Mus musculus*. J. Egypt Soc. Parasitol., 34: 45-64.
- Frenkel JK, Escajadillo A (1987). Cyst rupture as a pathogenic mechanism of toxoplasmic encephalitis. Am. J. Trop. Med. Hyg., 36: 517–522. https://doi.org/10.4269/ajtmh.1987.36.517
- Friedewald WT, Levy RI, Fredrickson DS (1972). Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. Clin. Chem., 18: 499–502. https://doi.org/10.1093/clinchem/18.6.499
- Gharadaghi Y, Shojaee S, Khaki A, Fathiazad F, Khaki A, Ghdamkheir E, Rouhaninia M (2012). Antiprotozoal effect of *Allium cepa* on acute renal failure caused by *Toxoplasma gondii*. Afr. J. Pharm. Pharmacol., 6(10): 771-777. https://doi.org/10.5897/AJPP12.086
- Guo M, Dubey JP, Hill D, Buchanan RL, Gamble R, Jones JL, Pradhan AK (2015). Prevalence and risk factors for *Toxoplasma gondii* infection in meat animals and meat products destined for human consumption. J. Food Prot., 78(2): 457–476. https://doi.org/10.4315/0362-028X.JFP-14-328
- Hammouda NA, Amin SM, Khalifa AM, Abou NI, Gaafar MR, Nasr MA (2006). The use of ELISA and immunohistochemistry techniques for detection of T*oxoplasma gondii* antigen in tissues of experimentally infected mice. J. Egypt Soc. Parasitol., 36: 925-935.
- Hilali M, Romand S, Thulliez P, Kwok OC, Dubey JP (1998). Prevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in sera from camels from Egypt. Vet. Parasitol., 75: 269–271. https://doi.org/10.1016/S0304-4017(97)00181-7
- Ibrahim HM (2013). Seroprevalence of *Neospora caninum* antibodies in chicken samples from Delta Egypt using a recombinant NcSAG1 protein-based ELISA. Egypt J. Immunol., 20(1): 29-37.
- Ibrahim HM (2016). Use of indirect ELISA based on recombinant protein NcSAG1 for seroprevalence of *Neospora caninum* antibodies in sheep, ducks and pigeons from Egypt. Indian J. Comp. Microbiol. Immunol. Infect. Dis., 37 (1): 31-36. https://doi.org/10.5958/0974-0147.2016.00008.8
- Ibrahim HM, Abdel-Ghaffar F, Osman GY, El-Shourbagy SH, Nishikawa Y, Khattab RA (2016). Prevalence of *Toxoplasma gondii* in Chicken samples from delta of Egypt using ELISA, histopathology and immunohistochemistry. J. Parasit. Dis., 40(2): 485–490. https://doi.org/10.1007/s12639-014-0530-7
- Ibrahim HM, Bannai H, Xuan X, Nishikawa Y (2009b). *Toxoplasma gondii* cyclophilin 18-mediated production of nitric oxide induces Bradyzoite conversion in a CCR5-dependent manner. Infect. Immun. 77(9): 3686-3695. https://doi.org/10.1128/IAI.00361-09
- Ibrahim HM, Geba KM, Tawfic AA, El-Magd MA (2019). Camel milk exosomes modulate cyclophosphamide-induced oxidative stress and immuno-toxicity in rats. Food Funct., 10: 7523-7532. https://doi.org/10.1039/C9FO01914F
- Ibrahim HM, Huang P, Salem TA, Talaat RM, Nasr MI, Xuan X, Nishikawa Y (2009a). Prevalence of *Neospora caninum* and *Toxoplasma gondii* Antibodies in Northern Egypt. Am. J. Trop. Med. Hyg., 80(2): 263–267. https://doi.org/10.4269/ajtmh.2009.80.263
- Ibrahim HM, Mohamed AH, El-Sharaawy AA, El-Shqanqery HE (2017). Molecular and serological prevalence of *Toxoplasma gondii* in pregnant women and sheep in Egypt. Asian Pac. J. Trop. Med., 10(10): 996–1001. https://doi.org/10.1016/j.apjtm.2017.09.012
- Ibrahim HM, Nishikawa Y (2016). The impact of the host CCR5, TLR2 and TLR11 on the nitric oxide, IL-6, IL-12 production and parasite growth during *Toxoplasma gondii* infection. J. Protozool. Res., 26: 18-29.
- Ibrahim HM, Osman GY, Mohameda AH, Al-Selwi AGM, Nishikawa Y, Abdel-Ghaffar F (2018). *Toxoplasma gondii*: Prevalence of natural infection in pigeons and ducks from middle and upper Egypt using serological, histopathological, and immunohistochemical diagnostic methods. Vet. Parasitol. Reg. Stud. Rep., 13: 45-49. https://doi.org/10.1016/j.vprsr.2018.04.002
- Jiménez-Pelayo L, García-Sánchez M, Regidor-Cerrillo J, Horcajo P, Collantes-Fernández E, Gómez-Bautista M, Hambruch N, Pfarrer C, Ortega-Mora LM (2019). Immune response profile of caruncular and trophoblast cell lines infected by high- (Nc-Spain7) and low-virulence (Nc-Spain1H) isolates of *Neospora caninum*. Parasitol. Vectors, 12(1): 218. https://doi.org/10.1186/s13071-019-3466-z
- Luft BJ, Remington JS (1988). AIDS commentary. Toxoplasmic encephalitis. J. Infect. Dis., 157: 16. https://doi.org/10.1093/infdis/157.1.1
- Luft BJ, Remington JS (1992). Toxoplasmic encephalitis in AIDS. Clin. Infect. Dis., 15: 211–222. https://doi.org/10.1093/clinids/15.2.211
- Mahboub HD, Helal MA, Abd-Eldai MA, Abd El-Razek EM, Elsify AM (2013). Seroprevalence of abortion causing agents in Egyptian sheep and goat breeds and their effects on the animal's performance. J. Agric. Sci., 5(9): 92-101. https://doi.org/10.5539/jas.v5n9p92
- Nishikawa Y, Ibrahim HM, Kameyama K, Shiga I, Xuan X (2011). Host cholesterol synthesis contributes to growth of intracellular *Toxoplasma gondii* in macrophages. J. Vet. Med. Sci., 73(5): 633-639. https://doi.org/10.1292/jvms.10-0496
- Portugal LR, Fernandes LR, Cesar GC, Santiago HC, Oliveira DR, Silva NM, Silva, AA, Lannes-Vieira J, Arantes RME, Gazzinelli RT, Alvarez-Leite JI (2004). Infection with *Toxoplasma gondli* increases atherosclerotic lesion in Apo E-deficient mice. Infect. Immun., 76: 6571-5676.
- Rimland D, Guest JL, Hernandez-Ramos I, Del Rio C, Le NA, Brown WV (2006). Antiretroviral therapy in HIV-positive women is associated with increased apolipoproteins and total cholesterol. J. Acquir. Immun. Def. Synd., 42: 307-313. https://doi.org/10.1097/01.qai.0000220164.72113.12
- Stockham SL, Scott MA (2002). Fundamentals of veterinary clinical pathology USA, Iowa State Press. pp. 251-277.

• Suzuki N (1971). Biochemical observation on rats infected with *Toxoplasma gondii*. Res. Bull. Obihiro. Univ., 7: 15-25.
• Tremblay AJ, Morrissette H, Gagné J-M, Bergeron J, Gagné C, Couture P (2004). Validation of the Friedewald formula for the determination of low-density lipoprotein cholesterol compared with β-quantification in a large population. Clin. Biochem., 37(9): 785–790. https://doi.org/10.1016/j.clinbiochem.2004.03.008