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Molecular and Serological Identification of *Neospora caninum* and The Associated Biochemical Changes in Egyptian Dairy Cattle.

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Abstract

FOR detecting *N. Caninum* in dairy cows and their associated biochemical and inflammatory markers, 77 blood samples were collected from animals were subjected for serological and molecular testing. Myeloperoxidase (MPO), interlukin-10 (IL-10), catalase, nitric oxide (NO), malondialdehyde (MDA), calcium and phosphorus were compared in serologically negative animals with sensitivity/specificity <30 % with those serologically positive chronic (S/P >40% and <100 %), and serological positive acute (S/P>100). Results indicated that 21animals were seropositive (27.27%). From the seropositive animals 10 (47.62%) were considered as chronic having S/P of 63.59±11.49 and 11 (52.38%) were considered acute having S/P of 200.59±71.78. Only, one suspected animal by serological (1.29%) testing was detected positive by PCR. Seropositive chronic cows had high (P>0.05) MPO but seropositive acute case had the highest IL-10 and phosphorus (P<0.01) associated the lowest calcium (P<0.05) and calcium phosphorus ratio. In conclusion, serological test can be used to distinguish between serological acute and chronic positive cases while molecular test can be used to confirm the infection in suspected cases. Additionally, levels of IL-10, calcium, phosphorus, or calcium/phosphorus ratio can act as biomarkers for acute neosporosis in dairy animals.

Keywords: Bovine neosporosis; ELISA; PCR; IL-10; inflammatory markers; minerals.

Introduction

Neospora caninum (*N. Caninum*) as many intracellular parasites can causes worldwide economic losses in dairy and beef cows due to abortion and the losses in neo-born calves or those congenitally infected [1]. The absence of zoonotic to human being decreased the veterinarian interest to control the spread of the parasite and using medication to date [2]. It also causes abortion in sheep and goats [3]. In buffaloes, *N. caninum* is associated with early embryonic death, abortion, and retained fetal membranes [4, 5]. The prevalence *N. caninum* decreases in the extensive farming system, the presence of sheep and goats, use of rodent control, the beef cattle, and the normal buffalo cows compared to those in the semi-intensive farming system, dairy cows, the absence of sheep and goats, after purchasing animals, and milk cooling system in dairy buffaloes [6]. Aborted *N. canium* sero-positive dairy cows showed high fertility one-month after abortion [7]. Abortion in sero-positive neospora infected dairy cows occurred in the first five months of pregnancy that conceived during the first month after abortion and required lower inseminations for conception compared to sero- negative dairy ones the first month after the second lactation [7].

The PCR is a more sensitive and specific diagnostic method than other methods, and it is less susceptible to autolysis or postmortem alterations. Furthermore, it can be utilized to detect N. caninum DNA in various fetal fluids, embryonic tissues, brain, spinal cord, feces, and even oocysts in the ultimate host's feces [8]. *N. caninum* with prevalence from 18.1 to 45.4% was detected by PCR in 11 pregnant, postpartum, and aborted buffalo cows [9]. In the blood serum, ELISA depended on the preparation of recombinant Dense Granule Protein for its detection in goats [10] and species-specific antigens were used

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to detect *N. caninum* in bovine [11]. According to Nematollahi *et al.* [12], serological assays like ELISA are often used assays for routine diagnosis of *N. caninum* infection. However, employing serological assays alone to confirm the diagnosis is insufficient. The most often used serological method is indirect ELISA, however there may be cross-reactivity with other protozoa such Sarcocysts and *T. gondii* [13]. Using ELISA, 16.2% of the dairy farms in Switzerland's had at least one seropositive cow [14].

Several cytokines are getting either increase or decrease in response to bacterial or parasitic infection. Experimentally, the compensated decrease in interlukin-12 (IL-12) by the injection of recombinant murine IL-12 attenuated infected mice encephalitis and brain load of the parasite [15]. Nitric oxide is one of the inflammatory modulation biomarkers for the parasite restriction and for modulating inflammatory responses [16].

This study aimed to estimate the prevalence of *Neospora caninum* in the blood of naturally infected Holstein Friesian cows using ELISA technique and the confirmation of infection by PCR with the evaluation of some the biochemical and mineral changes in response to *N. caninum* infection.

Material and Methods

Animals and blood sampling

Before conducting this investigation, the ethical approval was obtained (number 2471022023) by the National Research Centre Animal Care and Use committee. Multiparous Holstein Friesian Dairy cows (n= 77) belonged to a private dairy farm at Sadat city (Menoufia governorate). Cows had a history of repeat breeding. Blood and serum samples were collected for the detection of *Neospora caninum* DNA and antibodies respectively.

Neospora caninum Antibody Test Kit (IDEXX, USA) for detecting antibodies against *Neospora caninum* (*N. caninum*) in serum and plasma samples from ruminants was used. The absorbance of the samples and controls was read at 540 nm wave length. The equation used for the calculation of the negative control cases (mean NC)/ positive (Mean PC):

Mean negative control=the addition of the absorbance of the two negative standards (duplicate readings) then dividing them by two.

Mean positive control=the addition of the absorbance of the two positive standards (duplicate readings) then dividing them by two.

For determining the samples: S/P % (Sample absorbance - Mean NC) / (mean PC - Mean NC) ×100.

Samples of animals having S/P < 30 are considered negative but those having SP ≥ 40 are considered positive. Samples having S/P ≥ 30 and <40 are considered suspected and another sample should be collected and analyzed for the disclosing the animal status. Animals were classified negative (having S/P ≤ 30). Animals having S/P ≥40 to ≤100 are considered positive of low titer (chronic) and animals having S/P >100 are considered positive with high titer (Acute).

Blood biochemicals and immunological parameters

Bovine myeloperoxidase (double antibody sandwich enzyme linked immunosorbent assay; Sun Red, www.srbooo.com, China) with sensitivity of 0.738ng/ml and Bovine tumor necrosis factor α with sensitivity 2.5ng/ml were assayed. Interlukin-10 (IL-10) was assayed using Chongqing Biospes Co., LTD, www.biospes.com, China). The intra and inter assay precisions were <10% and <12%. Catalase, MDA, NO, calcium, and phosphorus commercial kits were assayed (Biodiagnostic, Egypt).

DNA extraction

DNA extraction from blood of Neospora seropositive samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) following the manufacturer's recommendations. DNA was eluted with 100 µl of elution buffer.

Molecular identification using conventional Polymerase Chain Reaction (PCR)

For the amplification of *N. caninum* DNA, Np21 (5'-GTGCGTCCAATCCTGTAAC-3') and Np6 (5'-CAGTCAACCTACGTCTTCT-3') (Vivantis, Malaysia) primers were used which amplify a fragment of 238 bp of *N. caninum* NC5 gene [17]. The PCR conditions were the following: the master mix of 25 µl contained 2 µl of DNA, 12.5 µl of 2x COSMO PCR RED Master Mix (Cat. W1020300X, Willofort Co., UK), 9.5 µl of sterile water, 0.5 µl of each primer (10 µM). The samples were amplified with a GS-96 gradient thermocycler (hercuvan, Malaysia). The reaction was initiated with a denaturation cycle of 95°C for 5 min, followed by 40 cycles at 95°C for 30 s, 50°C for 30 s, 72 °C for 1min and a final extension of 72 °C for 10 min. The PCR products were separated by electrophoresis on 1.5% agarose gel then photographed and analyzed by InGenius3 gel documentation system (Syngene, UK).

DNA analysis and phylogenetic tree building

The Gene JET^{TM} Gel Extraction Kit (K0691, Thermo Fisher, USA) was used to clean ten of the positive PCR products from *N. caninum* isolates that targeted the NC5 gene. The sequences were then run in Macrogen Company (Korea). Two-way sequencing

using the specific primers used in PCR served as a confirmation of the data's accuracy. The programs BioEdit 7.0.4.1 and MUSCLE were used to examine the nucleotide sequences acquired in this work. Using a neighbor-joining technique of the aligned sequences implemented in the application CLC 6, the obtained sequences were aligned with reference sequences genes of *N. caninum*.

Preparation of tissue samples

Tissue samples associated with blood samples were collected from nine cattle in the slaughterhouse of the same region (Uterus, liver, Lung and kidney) and immediately part is placed in fixative solution of 10% formalin to preserve its structure and other part preserved at -80 C. Positive tissue samples for *neospora* by PCR were subjected to dehydration by placing in series of alcohol solutions the dehydrated tissue is cleared in xylene. Then the tissue embedded in paraffin Wax and sectioned into thin sections (4-5 micrometers) the tissue sections were stained with hematoxylin and eosin (H&E) [18].

Statistical analysis

Data are presented as mean ± SD (standard deviation). Simple one-way ANOVA was used to differentiate between sero-negative (S/P<30), chronic sero-positive cows (S/P positive >40 and <100, chronic), and acute sero-positive (S/P>100) using IBM-SPSS 20.0 (2016). Duncan's Multiple Range test was used to differentiate between significant means at P<0.05.

Results

Using ELISA for the detection of seropositive cows indicated that the percentage of seropositive cows was 27.27% (21 out of 77samples). Seropositive chronic cases were 10 out of 21 (47.62%) that had a percentage of 12.99% from the total cases. Seropositive acute cases were 11 out of 21 positive cases (52.38%) and 11 out of 77 total cases (14.29%). One cow with a percentage of 1.297% (1 out of 77 samples) was suspect with S/P of 35.07%. Seronegative cases had a percentage of 71.43% (55 out of 77 cases).

Mean S/P of cows with different serological identification is presented in table (1). Seronegative cases had the lowest (P<0.0001) mean S/P of 14.93±8.85% (Reference <30). Acute cases had 200.59±71.78 S/P% (Kit Reference >40. Chronic cases had S/P of 63.59±11.49% (Kit Reference >40).

The concentrations of myeloperoxidase (MPO) tended to increase (P=0.08) in chronic seropositive cows (42.78±22.37) compared to negative controls (39.19±23.76) but is significantly (P<0.05) higher than acute sero-positive dairy cows (27.27±1.698; Table 1). Interlukin-10 increased (*P*=0.009) in sero-positive dairy cows acute (92.82±21.60) and chronic (72.97±40.23) cows compared to seronegative cows (59.01±30.16). The activity of catalase enzyme insignificantly decreased in the sero-positive acute and chronic cows compared to the sero-negative ones. NO tended to slightly increase (P=0.055) in sero-negative cows compared to seropositive acute cows or seropositive chronic ones. MDA levels are nearly similar (*P*>0.05) in sero-negative (22.33±0.79) and sero-positive cows acute (22.35±0.66) or seropositive chronic cows (22.43±0.49). Phosphorus showed elevated (*P*<0.01) levels in the sero-positive acute animals (5.09±1.25) compared to either sero-positive chronic cows (4.33±0.79) sero-negative cows (4.48±0.96). Calcium levels declined (P=0.014) in seropositive acute cases (10.28±1.21) compared to sero-positive chronic cows (10.95±0.60) with no significant difference compared to seo-negative (10.57±0.89). Calcium/phosphorus ratio reached the lowest value (*P*=0.023) in the seropositive acute cows (2.20±0.62) compared to the sero-negative (2.47±0.58) cows and seropositive chronic cases (2.63±0.61).

Molecular identification using conventional Polymerase Chain Reaction (PCR)

All the 21 seropositive samples were also positive using conventional PCR with the predicted PCR product (328 bp; Fig.1). The serologically suspected sample is considered positive in the PCR with a total percentage of 28.57% (22/77).

The sequencing of PCR products obtained from ten blood samples of Multiparous Holstein Friesian Dairy cows showed that the amplified sequence was *N. caninum* specific. As all the sequences were 100% identical, only one of them was deposited in the GenBank with accession number of **PP997253**. The results demonstrated our sequence shared 100% similarity with *N. caninum* deposited in GenBank. Phylogenetic trees (Fig. 2) of our sequences showed high similarity (100%) with the sequence of *N. caninum* isolated from cattle blood and cattle fetal brain in Egypt (PP708713 and OR939832), bovine abortion in Austria (AF190701), dairy cows in Slovakia (GU300174), cow brain in South Korea (FJ464412), buffalo in Brazil (AY497045), sheep blood (OQ054167) and placenta (OP574221) in Iraq, goat blood (PP265560) and milk (PP265563) in Iraq, and chicken brain in brazil (EU073600).

Four uterine tissue samples showed dilatation of uterine glands with severe degeneration of lining epithelium. Some uterine tissue samples revealed marked edema and periglandular mononuclear inflammatory cells infiltrations. Six out of nine samples showed severe degeneration and desquamation of endometrium lining epithelial cells Fig. (3,4). Liver Hepatocytes showed vacuolar

degeneration associated with inflammatory cells infiltration in portal area Fig. (5). The kidney of the severe degeneration and desquamation of lining epithelium of some renal tubules. Inter tubular diffuse inflammatory cells infiltration were seen. Lungs showed Dilatation of alveoli and rupture of alveolar wall forming giant alveoli. In some cases, thickening of alveolar wall.

Discussion

Serological testing or molecular assaying indicating the presence of *N. caninum* in cases of abortion are not conclusive diagnosis rather than the detection of specific lesions or the protozoa in the aborted materials unless other factors causing abortion should be investigated [19, 20].

Neosporosis gained attention as one of the most common infections causing abortion in dairy cattle worldwide and puppies' mortalities [21]. Great economic impact of N. caninum infection due to decreased fertility, abortion, and still birth of newborn animals with evidence of vertical transmission in slaughtered animals [22, 23]. The clinical symptoms of *N. caninum* have been observed in water buffaloes, sheep, goats, camel, and horses [3, 21, 24-25]. Furthermore, antibodies have been detected in the sera of Coyotes, foxes, and felines [26]. The prevalent reproductive issue that causes significant financial loss in the sheep and cattle industries due to abortion brought on by *N. caninum* [27, 28]. The presence of the parasite in the sheep and goats aborted feti confirmed its transplacental access to the feti and its role in inducing abortion [3]. In the current study, all the serologically positive samples for *N. caninum* plus the suspected animal's sample were positive by PCR. This contradicted with Reyes-Sandoval et al. [9] who reported that not all serologically positive samples collected from calves for *N. caninum* were confirmed by detecting *N. caninum* DNA isolated from their blood suggesting that blood works as a transit factor for Neospora tachyzoites to reach different tissues [29]. In this study, PCR was positive in 22 out of 77 animals (28.87%). Earlier studies reported that the likelihood of presenting antibodies and/or finding *N. caninum* DNA increases with increasing female age and number of births in buffalo and dairy cattle [30, 31].

In contrast to the high prevalence of *N. caninum* in dairy cows in this study by either serological identification using ELISA or molecular identification, the prevalence of *N. caninum* in Italian buffaloes was as low as 20.2% (25 out of 124) and 19 out of 124 animals (15.3%) were positive to both *Toxoplasma gondii* and *N. caninum* in buffaloes suffering from abortion, retained placenta, and embryonic death [4]. Other report found a prevalence of 19.1%; that was obtained from 26 neospora seropositive cases out of 136 buffaloes samples collected from 14 herds [6]. Higher prevalence reached 45% were recorded in pregnant and aborted buffaloes [9]. In sheep, goats, and cattle, the prevalence determined by molecular identification ranged from 0.0 to 20.5% and early pregnant animals showed the highest prevalence (32%) among seropositive animals [32]. The noteworthy was the elevated *N. caninum* prevalence could be due to absence of effective control measures such as improved biosecurity, screening, and isolation of new animal introductions. This was likely because the players in the animal health industry were not aware that the conditions existed in Egyptian dairy cattle and, as a result, were not well-informed about the consequences of these diseases hosts [33]. Moreover, increasing interaction, either directly or indirectly, with nearby endemic areas; and exposure to definitive hosts or intermediate hosts [34]. A comparison of the *Nc5* gene sequence obtained herein with partial and complete genomes pointed to the closest proximity with bovine and ovine origin from Egypt, Iraq and Brazil. It is important to note that the sequences displayed in Figure 2 come from a variety of intermediate hosts, including bovines and buffaloes, as well as definitive hosts, like dogs and wolves. The results of phylogenetic analysis indicted that *N. caninum* from different hosts and geographical areas are genetically diverse.

Myeloperoxidase (MPO) is a member of the mammalian peroxidase family and is known to be effective in killing bacteria and its levels increased during uterine infection and enhanced systemic inflammation [35]. This study reported a significant increase in the levels of MPO in seropositive chronic cows compared to seropositive acute cows and this could be a marker for uterine inflammation and predicted abortion. In pregnant women, MPO expression increased in the blood plasma and the placental syncytiotrophoblast cells and the extravillous trophoblast cells [36].

The current study showed increase of IL-10 in acute seropositive cows with mean S/P of 200.59±71.78. Interleukins are well known biological markers of inflammation that increase in response to infection. Different virulence of N. caninum infection showed different biological responses where its ability to invade, survive, proliferate, and modulate the host cell immune responses usually connected with specific gene expression in the tachyzoite stage [37]. In female mice used as *N. caninum in vivo* model, both toll like receptor 2 and 3 increased to stimulate the increase in the expression of interlukin-12p40 [2] which is in agreement with the increase of IL-10 in acute *N. caninum* seropositive cows with mean S/P of 200.59±71.78. Experimentally infected pregnant

heifers with high or low virulent neospora tachyzoite stage showed different biological responses, abilities to invade, survive, proliferate, and modulate the host cell immune responses which was linked to the specific gene expression in the tachyzoite stage [38]. In agreement with the increase of IL-10 in seropositive cows of this study, pregnant heifers experimentally infected with low and high virulent *N. caninum* Tachyzoites showed increased IFγ and IL-4 earlier 6 days post infection in the high virulent one- and 9-days post infection in the low virulent one [38]. Moreover, pro-inflammatory cytokines such as IFNγ, IL-2 and TNFα are detrimental to the maintenance of pregnancy and their increase in the cows with high S/P could help in predicting abortion and starting the treatment to avoid it [39, 40].

However, the current study could not recommend the use of Interferon or interleukins for the treatment of naturally infected dairy cows due to the absence of data about chronicity of the disease in farm animals, the high costs, side effects on fetal life, and the higher the doses required for trying it in large animals compared to experimentally controlled infected and treated mice with IL-12p40 via inducing a paracrine effect on infected macrophages to control the parasite proliferation [2].

The current study showed non-significant changes in oxidative markers (catalase, MDA, NO) in infected dairy cows and this may be referred to the ability of *N. caninum* to withstand the host response to infection by neutralizing the oxidative stress via secreting her own antioxidants. This suggestion was confirmed during *Trypanosoma cruzi* (*T. cruzi*) *in vivo* experimental infection model by detecting increased hydrogen peroxide production by the peripheral blood monocytes and macrophages [41]. In agreement with our results, *T. cruzi* infection did not result in oxidative response in the host body [42]. The absence in the oxidative stress in our dairy cows could be referred to the ability of N. caninum to withstand the host response to infection by neutralizing the oxidative stress by secreting her own antioxidants [43]. This would be the reason for the absence of variation in catalase, MDA, nor NO in our dairy cows naturally infected even when seropositive animals were subdivided into acute or chronic depending on the S/P percentages. In contrast to the naturally infected dairy cows of this study, the increase in ROS production and NO observed in experimentally infected female mice [2] could be attributed to the high parasite count injected intraperitoneally and the shortest experimental interval for five days. This oxidative stress was referred to the secretion of interferon-gamma (IFN-γ) which activated the inducible nitric oxide synthetase enzyme [16, 44]. During *N. caninum* infection, the treatment of infected mice with IL-12p40 increased the production of NO from their macrophages [2].

This study recorded low calcium and calcium/ phosphorus ratio and high phosphorus in seropositive acute cows compared to seropositive chronic cows associated an increase of phosphorus in seropositive acute cows compared to seronegative controls. This decrease in calcium and the imbalance between calcium and phosphorus could be related to the response of the infected animals in the secretion of two calcium-dependent protein kinase 1 inhibitors to interfere with vertical transmission of *N. caninum* [44]. However, the decrease in calcium in acute seropositive animals of this study agree with the sustained increase of Ca^{++}-related fluorescence signals in both *N. caninum*-infected host cells and non-infected controls but the treatments with the calcium ionophore A23187 at 24- and 42-h post-infection induced a fast and sustained increase in Ca++ signals in parallel to tachyzoite egress [45].

In the histopathological sections, the intensive inflammatory cells infiltration of uterine tissue could be adaptive immune response. In several studies, the liver lesion such as inflammatory cells infiltration mainly lymphocytes of portal area is related to N. caninum as one of the suspected causative agents of abortion [46]. The pulmonary lesion in this study as thickening of some alveolar wall as predominant lesion agreed with the previous findings [47].

Conclusion

Both serological and molecular detection can be used for the detection of *N. caninum* in blood of animal showing abortions in addition to the detection of tachyzoites. High S/P (>100) indicate acute infection but low positive ones >100 indicate chronic cases. The decrease of calcium concentrations, myeloperoxidase, and interlukin-10 may predispose to systemic inflammation and abortion in pregnant animals.

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Declaration of Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical of approval

The ethical approval was obtained (number 2471022023) by the National Research Centre Animal Care and Use committee.

TABLE 1. Serological and biochemical findings for *N. caninum* in blood samples of dairy cows

Parameter	Negative	Acute	Chronic	P-value
N	**55**	**11**	**10**	
Neospora S/P	14.93±8.85[a]	200.59±71.78[c]	63.69±11.49[b]	0.0001
MPO ng/ml	39.19±23.76[ab]	27.27±1.698[a]	42.78±22.37[b]	0.08
IL-10 pg/ml	59.01±30.16[a]	92.82±21.60[b]	72.97±40.23[ab]	0.009
MDA nmol/ml	22.33±0.79	22.35±0.66	22.43±0.49	NS
Catalase U/ml	64.73±70.74	50.37±43.27	58.46±42.65	NS
NO µmol/L	50.62±3.35	49.42±4.16	49.29±3.84	0.055
Calcium (Ca) mg/dl	10.57±0.89[ab]	10.28±1.21[a]	10.95±0.60[b]	0.014
Phosphorus (P) mg/dl	4.48±0.96[a]	5.09±1.25[b]	4.33±0.79[a]	0.006
Ca/P	2.47±0.58[b]	2.20±0.62[a]	2.63±0.61[b]	0.023

Mean ±SD of myeloperoxidase (MPO, ng/ml), Interlukin-10 (IL-10, pg/ml), catalase (U/ml), malondialdehyde (MDA nmol/ml), nitric oxide (NO, µmol/L), Phosphorus (mg/dl), calcium mg/dl), and calcium /phosphorus ratio

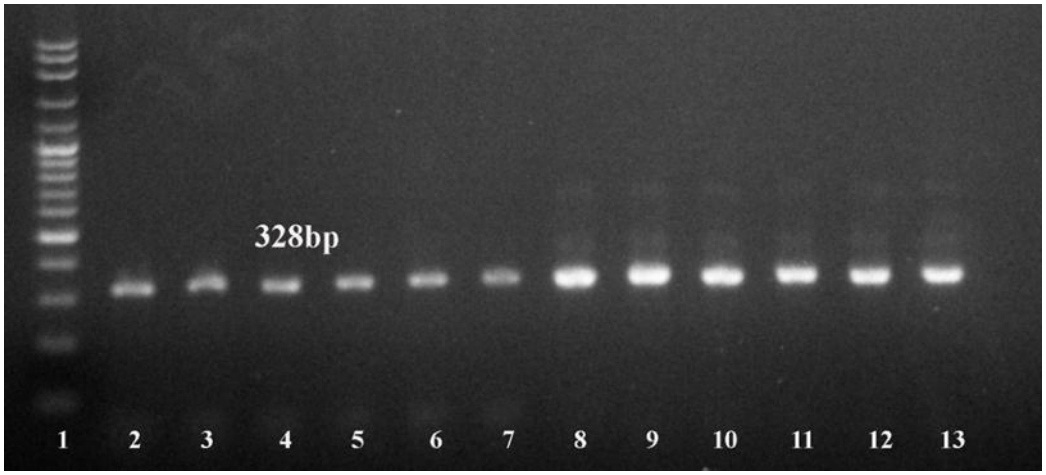

Fig.1. Agarose gel electrophoresis of PCR product amplified from *N. caninum* (328 bp). Lane 1, 100bp DNA ladder; Lanes, 2-13, positive samples.

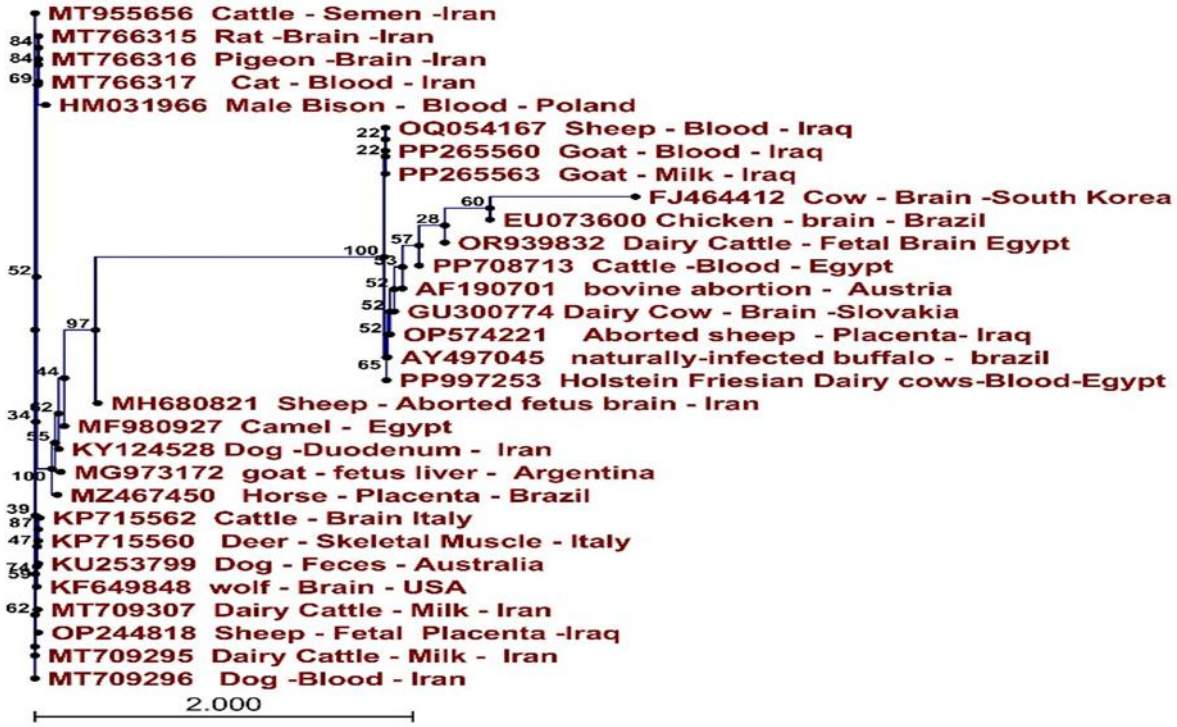

Fig. 2. Phylogenetic tree of *N. caninum* isolates from Egypt and the other isolates deposited in GenBank BLAST

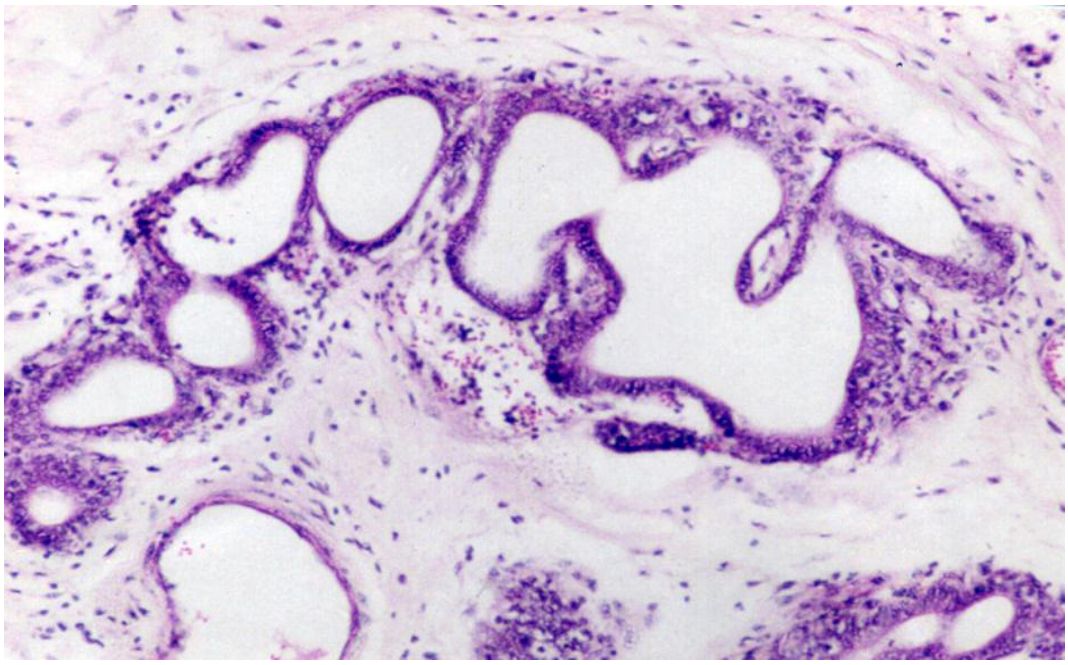

Fig. 3. Uterus of cattle with positive neospora showed severe dilatation of uterine glands lumen and diffuse edema (H&E, X100).

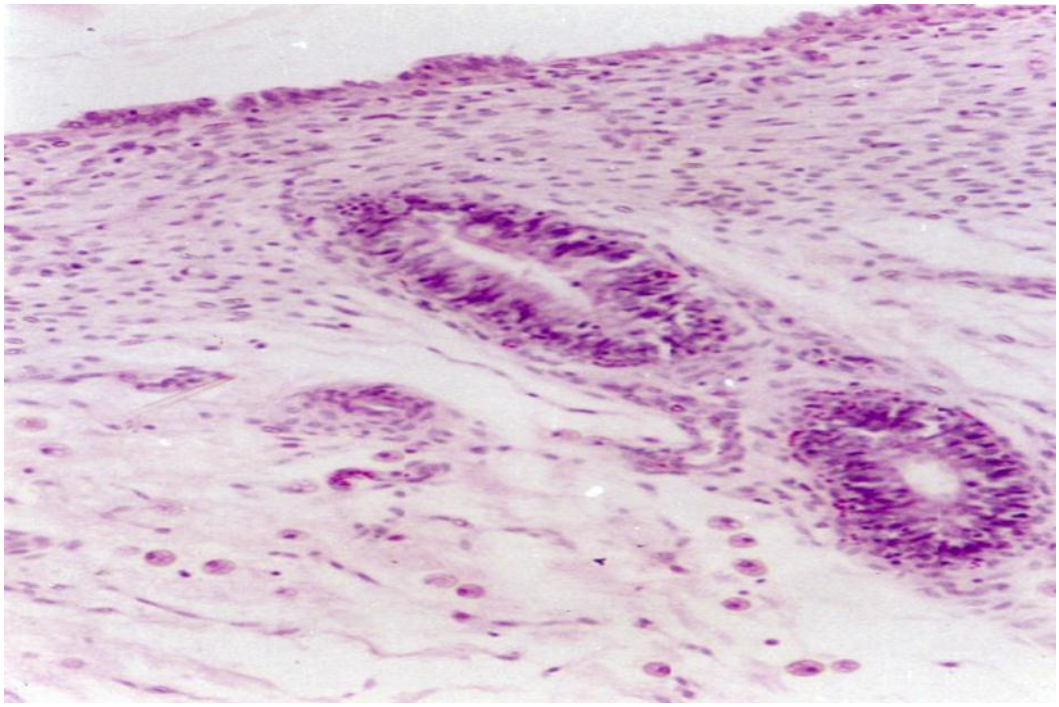

Fig. 4. Uterus of cattle positive with neospora revealed degeneration and partial desquamation of lining epithelium of endometrium associated with periglandular mononuclear inflammatory cells infiltration (H&E, X100).

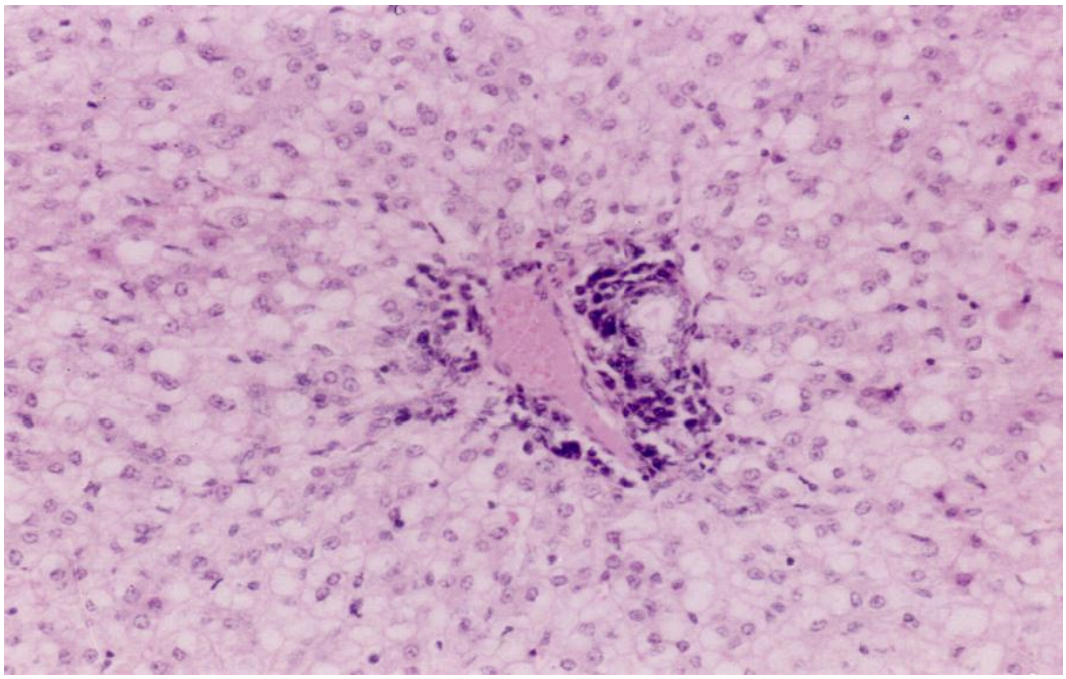

Fig. 5. Liver of cattle positive with neospora revealed distortion of hepatic cord and vacuolar degeneration of hepatocytes associated with inflammatory cells infiltration in portal area (arrow) (H&E, X100).

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تشخيص النيوسبورا السيرولوجي والجزيئي مع ارتباطها بالمؤشرات البيوكيميائية في الابقار الحلابة بمصر

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الملخص

لتشخيص النيوسبورا كانينم في الابقار الحلابة و المؤشرات البيوكيميائية و الالتهاب تم تجميع عدد 77 عينة دم من ابقار الهولشتين فرزيان و تم تشخيصها بالطريقة السيولوجية و الجزيئية و تم قياس انزيم الميلوبياوكسيديزو الانترلوكيم -10 و انزيم الكاتاليز و اكسيد النيتريك و المالون ثنائي الهيدروجين و الكالسيوم و الفسفور و تم مقارنتها في الحيوانات السالبية سيرولوجيا و التي كان بها الحساسية / النوعية اقل من 30 و اللايجابية و صنفت كمزمنة الاصابة و كان مستوى الحساسية/ النوعية اكثر من 40 و اقل من 100 و الايجابية و صنفت حادة الاصابة و كان مستوى الحساسية/النوعية اكثر من 100. اظهرت النتائج ايجابية الاختبار السيرولوجي لعدد 21 حيوان بنسبة 27.27% و كان منهم 10 حيوانات صنفت مزمنة الاصابة بنسبة 47.62% و كان مستوى الحساسية/النوعية 11.49±63.59 و 11 حيوان صنفت على انها حادة الاصابة بنسبة 52.38% و كان مستوى الحساسية/النوعية 71.78±200.59 و تم استبعاد حيوان واحد مشتبه به من باقي التحاليل بنسبة 1.29% رغم ثبوت ايجابيته بالاختبار الجزيئي. و اظهرت الحيوانات مزمنة الاصابه مستوى عالي ($P>0.05$) من انزيم الميلوبياوكسيديزبينما تلك حادة الاصابة اظهرت اعلى مستوى من الانترلوكين 10 و الفسفور و اقل مستوى للكالسيوم و نسبة الكالسيوم للفسفور.و يمكن الاستنتاج ان الاختبار السيرولوجي يمكن استخدامه لتشخيص الاصابة بالنيوسبورا كانينم و ان يستخدم الاختبار الجزيئي لتاكيد الحالات المشتبه بها و السلبية الاصابة و تحديد مستويات الانترلوكين-10 و الكالسيوم و الفسفور كمؤشرات بيولوجية لتحديد الاصابات الحادة من المزمنة.

الكلمات الدالة : نيوسبورا ، اختبار الاليزا ، تفاعل البلمرة المتسلسل ، انترلوكين ١٠ ، دلالات الالتهابات ، المعادن.