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Parasitic view of abortion syndrome in dairy cattle and buffaloes

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Abstract

Neospora caninum is a worldwide parasite that is considered a cause of bovine abortion leading to severe economic losses in its industry. Thus, we conducted this study to detect antibodies in dairy cattle and buffaloes in some localities of Assiut governorate, Egypt. The prevalence of *N. caninum* antibodies of both serum and milk by ELISA were 5.4% and 57.1% in dairy cattle and 19.4% and 47.2% in buffaloes respectively. All animal serum samples positive to *N. caninum* antibodies were positive in milk. Significant differences of prevalence between two species were recorded by serum ELISA. The prevalence of *N. caninum* antibodies of dairy cattle and buffaloes according to age by ELISA were 57.1% and 58.3% with age ≤5 years and were 57.1% and 41.7% with age >5 years respectively. The prevalence of *N. caninum* antibodies of dairy cattle and buffaloes according to housing system by ELISA were 77.8% and 60% of farm rearing and 20% and 38% of household rearing respectively. There were very high significant differences of prevalence between two housing groups in cattle. The prevalence of *N. caninum* antibodies of dairy cattle and buffaloes in serum according to pregnancy status by ELISA were 0% and 18.8% of pregnant animals while were 6.7% and 20% of non-pregnant ones respectively. The prevalence of *N. caninum* antibodies of dairy cattle and buffaloes in milk according to pregnancy status by ELISA were 18.2% and 43.8% of pregnant animals and 66.7% and 50% of non-pregnant ones respectively. There were high significant differences of prevalence of non-pregnant cattle than pregnant ones. The present results proved high prevalence of *N. caninum* antibodies in dairy bovines in some localities of Assiut governorate and this necessitates the application of more effective strategies to control this infection. In addition, it revealed that ELISA is a useful tool for detection of *N. caninum* antibodies in bovine milk samples more than serum samples.

Keywords: *Neospora caninum, cattle, buffaloes, ELISA, Assiut.*

Introduction

Neosporosis is a disease caused by the obligate intracellular protozoan parasite *Neospora caninum* (*N. caninum*). Infection mainly occurs in cattle and dogs and less frequently in other animals such as goat, sheep, horse and deer (**Dubey *et al.*, 2007 and Jin *et al.*, 2017).** Abortions caused by *N. caninum* infection in buffaloes have also been demonstrated (**Guarino *et al.*, 2000 and Gennari *et al.*, 2005**).

N. caninum is a major cause of abortion in cattle and causes severe neuromuscular disease in dogs with a worldwide distribution (**Reichel *et al.*, 2007 and Dubey and Schares, 2011**). Abortion occurs during the 5^{th}-6^{th} month of gestation, sporadically, endemically or epidemically (**Schares *et al.*, 1998**).The rate of reproductive problems in cattle has been increasing over years and causes many economic problems in the bovine industry (**Yoo, 2010**), like repeated abortions, stillbirths, temporary anestrus, less milk and beef production with premature culling and vertical infection of calves which may born dead or alive with clinical signs or apparently healthy but with persistent chronic infection that can be later transmitted by females to their progeny (**Dubey *et al.*, 2007; Dubey and Schares, 2011 and Reichel *et al.*, 2013**). Other clinical signs have been identified in calves less than 4 months of age and include neurologic manifestations, locomotor disturbances and ocular and cerebral anomalies (**De Meerschman *et al.*, 2005**).

Vertical infection is generally considered the primary mode of transmission between cattle but is insufficient to sustain the infection in a herd (**Davison *et al.*, 1999**). Thus horizontal infection is considered complementary to vertical transmission and enables the introduction of new infections into naive herds by ingestion of contaminated food or water with sporulated oocysts. Naturally infected cows can exhibit a rate of endogenous transplacental transmission as high as 95% and this may occur during successive gestations (**Davison *et al.*, 1999 and-Dubey *et al.*, 2007**). Postnatal transmission by maternal milk has also been demonstrated (**Dubey *et al.*, 2007 and Dubey and Schares, 2011**).

Many serological tests, among which an enzyme-linked immunosorbent assay (ELISA) are widely used for detection of *N. caninum* antibodies and are commercially available (**Guido *et al.,* 2016)**.

The routine diagnosis for *N. caninum* infection in cattle is based on detection of specific antibodies in serum samples, but also milk samples can be used for lactating cows. Whole and skim milk samples were analyzed with a commercial serum ELISA test and both were equally suited as a screening tool (**Enachescu *et al.*, 2014)**. The antibodies found in milk are selectively transported from the serum into the mammary gland and the IgG is the primary immunoglobulin class for bovine milk **(Hurley and Theil, 2011)**.

This study aimed to investigate the prevalence of *N. caninum* specific antibodies in serum and milk of dairy cattle and buffaloes by using ELISA in Assiut governorate. Accurate prevalence data are required to develop an effective control strategy for bovine neosporosis.

Materials and Methods

1) Collection and preparation of milk and blood samples:

A total of 184 milk and serum samples of 92 dairy bovines (cattle, n=56; buffaloes, n=36) were collected randomly from the same animals with age ranged from 3 to 15 years, divided into two age groups: ≤5 years and >5 years. Out of the examined 92 bovines, 11 were pregnant cattle and 16 were pregnant buffaloes as well as two aborted animals; one aborted buffalo in the 8^{th} month and other one aborted cattle in 6^{th} month.
The bovines were randomly selected from Assiut city and different rural regions (Abnoub, Sahel Seleem, Al-Qusiya, Al-Fath and Abuteeg) belonging to various livestock owners and farms in Assiut governorate, Egypt in the period from May 2019 - September 2019.

(A)**Milk samples:** about 5ml milk were taken manually from each animal after disinfection of the teats with 70% ethyl alcohol and collected in clean dry and sterile test tubes. Samples were kept in cold conditions until arrival to the laboratory. Milk samples were centrifuged at 2000 rpm for 20minutes and the interface between the lipid layer and the pelleted cellular debris were rapidly frozen at -20^{o}C until used for serological examination (**Grundy *et al.*, 1983)**.

(B) Blood samples: about 5ml blood were collected from jugular vein of cattle and buffaloes into clean dry glass tubes without anticoagulant and transported to the laboratory in cold conditions. Sera were separated by centrifugation at 3000 rpm for 15 minutes then labeled and stored at –20°C until used (**Fereig *et al.* 2016).**

2) Serological examination:

Serological investigation of the collected sera and milk for the presence of anti-*Neospora caninum* antibodies was done using indirect enzyme linked immunosorbent assay (ELISA) kit. The serological results were grouped in classes and tabulated on the variables of animal species, age, rearing system (household, farm) and pregnancy status.

Detection of *N. caninum* antibodies in serum and milk samples by indirect ELISA:

The serum and milk samples were analysed for the presence of IgG antibodies specific for *N. caninum* using a commercially available indirect ELISA kit in serum or milk [ID Screen® *Neospora caninum* Indirect ELISA kit for the detection of anti-*Neospora caninum* antibodies in serum or milk (ID.VetInnovative Diagnostics Louis Pasteur. Grabeis, France) NCS ver 0818 EN, LOT: F34] according to the manufacturer's instructions. All control tests were performed in duplicate. The diluent, wash solution and dilution buffer were primed according to manufacture instruction. The optical density (OD) values were read with ELISA reader (Sunrise, TECAN) at a wave length of 450 nm within15minutes. The ELISA performed in Molecular Biology Research Center, Assiut University.

Antibody analysis:

For each sample, calculate the S/P percentage (S/P%) obtained by an equation provided by the manufacture.

$$S/P\% = \frac{\text{OD sample} - \text{OD negative control}}{\text{OD positive control} - \text{OD negative control}} \times 100$$

Serum samples with an S/P% > 40% were considered positive and those with S/P%≤40% were considered negative.

Milk sample with and S/P% > 25% were considered positive and those with S/P% ≤ 25% were considered negative.

3) Statistical analysis:

Differences in the prevalence of *N. caninum* infection according to species**,** age, rearing system (household, farm) and pregnancy status were determined using Chi square by the statistical **software SPSS** (Version 17; SPSS Inc., Chicago, USA) for data analyses. P value <0.05 was considered statistically significant (**Miroud *et al.*, 2019).**

Results

In the current study, the prevalence of *N. caninum* antibodies in dairy cattle was 57.1% (32/56) and in buffaloes were 47.2% (17/36) in some localities of Assiut governorate. No significant differences of prevalence between two species were recorded by ELISA. All animal serum samples positive of *N. caninum* antibodies were positive in milk. The prevalence of *N. caninum* antibodies of serum in dairy cattle and buffaloes was 5.4% (3/56) and 19.4% (7/36) by ELISA respectively. Also, the prevalence of *N. caninum* antibodies in milk of dairy cattle and buffaloes was 57.1% (32/56) and 47.2% (17/36) by ELISA respectively. Significant differences of prevalence between two species were recorded by serum ELISA, while no significant differences by milk ELISA (**Table 1**).

The prevalence of *N. caninum* antibodies of dairy cattle according to age by ELISA was 57.1% (16/28) with age equal or lower than 5 years and was 57.1% (16/28) with age more than 5 years. The prevalence of *N. caninum* antibodies of dairy buffaloes according to age by ELISA was 58.3% (7/12) with age equal or lower than 5 years and was 41.7% (10/24) with age more than 5 years. There were no significant differences of prevalence between two age groups (**Table 2 and 3**).

The prevalence of *N. caninum* antibodies of dairy cattle according to housing system by ELISA was 77.8% (28/36) of farm rearing and was 20% (4/20) of household rearing. There were very high significant differences of prevalence between two housing groups. The prevalence of *N. caninum* antibodies according to housing system of dairy buffaloes by ELISA was 60% (9/15) of farm rearing and was 38% (8/21) of household rearing. There were no significant differences of prevalence between two housing groups (**Table 4 and 5**).

The prevalence of *N. caninum* antibodies in serum of dairy cattle according to pregnancy status by ELISA was 0% (0/11) of pregnant cattle and was 6.7% (3/45) of non-pregnant cattle. The prevalence of *N. caninum* antibodies according to pregnancy status in serum of dairy buffaloes by ELISA was 18.8% (3/16) of pregnant buffaloes and was 20% (4/20) of non-pregnant buffaloes. There were no significant differences of prevalence of non-pregnant cattle and non-pregnant buffaloes than pregnant ones. The prevalence of *N. caninum* antibodies of dairy cattle in milk according to pregnancy status by ELISA was 18.2% (2/11) of pregnant cattle and was 66.7% (30/45) of non-pregnant cattle. There were high significant differences of prevalence of non-pregnant cattle than pregnant ones. The prevalence of *N. caninum* antibodies according to pregnancy status of dairy buffaloes in milk by ELISA was 43.8% (7/16) of pregnant buffaloes and was 50% (10/20) of non-pregnant buffaloes. There were no significant differences of prevalence between pregnant and non-pregnant buffaloes (**Table 6 and 7**).

The present study showed that the aborted buffalo in the 8th month was positive for *N. caninum* antibodies in both serum and milk samples. The 6th month aborted cattle was negative for *N. caninum* antibodies.

Table (1). Prevalence of *N. caninum* antibodies in serum and milk samples of dairy cattle and buffaloes by ELISA:

Species	No. of examined animals	serum samples		Chi-Square	P	milk samples		Chi-Square	P
		No. of positive	Prevalence (%)			No. of positive	Prevalence (%)		
Cattle	56	3	5.4	4.488	0.034	32	57.1	0.866	0.351
Buffaloes	36	7	19.4*			17	47.2		

*Significant differences (P<0.05)

Table (2). Prevalence of *N. caninum* antibodies according to age in dairy cattle by ELISA:

Age of animal	No. of examined animals	No. of positive	Prevalence (%)	Chi-Square	P
≤5 years	28	16	57.1	0.00	1
>5 years	28	16	57.1		
Total	56	32	57.1		

Table (3). Prevalence of *N. caninum* antibodies according to age in dairy buffaloes by ELISA**:**

Age of animal	No. of examined animals	No. of positive	Prevalence (%)	Chi-Square	P
≤5 years	12	7	58.3	0.891	0.345
>5 years	24	10	41.7		
Total	36	17	47.2		

Table (4). Prevelance of *N. caninum* antibodies according to housing system in dairy cattle by ELISA:

Housing system	No. of examined animals	No. of positive	Prevalence (%)	Chi-Square	P
Farms	36	28	77.8***	17.525	0.0000283
Household	20	4	20		
Total	56	32	57.1		

*** Very high significant differences (P<0.001)

Table (5). Prevelance of *N. caninum* antibodies according to housing system in dairy buffaloes by ELISA:

Housing system	No. of examined animals	No. of positive	Prevalence (%)	Chi-Square	P
Farms	15	9	60	1.684	0.194
Household	21	8	38		
Total	36	17	47.2		

Table (6). Prevelance of *N. caninum* antibodies in serum among dairy cattle and buffaloes according to pregnancy status:

Species	Pregnant			Non pregnant			Chi-Square	P
	No. of examined animals	No. of positive	Prevalence (%)	No. of examined animals	No. of positive	Prevalence (%)		
Cattle	11	0	0	45	3	6.7	0.774	0.379
Buffaloes	16	3	18.8	20	4	20	0.009	0.925

Table (7). Prevelance of *N. caninum* antibodies in milk among dairy cattle and buffaloes according to pregnancy status:

Species	Pregnant			Non pregnant			Chi-Square	P
	No. of examined animals	No. of positive	Prevalence (%)	No. of examined animals	No. of positive	Prevalence (%)		
Cattle	11	2	18.2	45	30	66.7**	8.484	0.004
Buffaloes	16	7	43.8	20	10	50	0.139	0.709

** High significant differences (P<0.001)

Discussion

Neospora caninum is one of the important reasons of reproductive problems in cattle. They cause various signs in animals like infertility, early embryonic death, abortion and stillbirth (**Adis *et al.*, 2018**). The annual costs due to losses caused by *N. caninum* were estimated to be equal between $1.298 and $2.380 billion/ year worldwide (**Reichel *et al.*, 2013**).

Unlike toxoplasmosis, neosporosis can cause repeated abortion in cattle **(Williams *et al.*, 2003).** Outbreaks of *Neospora*-associated abortion in herds can be caused by point source infection by the parasite or by reactivation of the parasite in chronically infected cows **(Paré *et al.*, 1997).** Vertical transmission of *N. caninum* happens during terminal stages of gestation (transplacental transmission with fetal infection) or postnatally by the transmission of tachyzoites via milk (transmammary transmission) **(Dubey *et al.*, 2007)**

In the current study, the prevalence of *Neospora caninum* antibodies was 57.1% and 47.2% in dairy cattle and buffaloes respectively by ELISA in some localities of Assiut governorate. In comparison to our results, nearly same result was recorded in Egypt by **Dubey *et al.* (1998)** who found that the seroprevalence of *N. caninum* was 68% of water buffalo. Lower results were reported from different regions in Egypt by **El-Ghaysh *et al.* (2003)** who found that the infection with *N. caninum* was 16.2% in cattle using direct agglutination test. Also, **Ibrahim *et al.* (2009)** found that the seroprevalence of *N. caninum* antibodies was 20.43% of cattle in delta, Egypt and **Fereig *et al.* (2016)** estimated prevalence of *N. caninum* of cattle was18.9% by using ELISA in southern Egypt, Sohag and Qena. Also, lower results were reported by **Gerges *et al.* (2018)** who revealed that *N. caninum* antibodies were detected in 29% of serum samples and 10% of milk samples in four governorates of Upper Egypt. They added that, the prevalence in El-Fayoum, Giza, Beni-Swief and El-Menia were 28%, 28%, 36% and 24%, respectively in serum by ELISA. This difference can be attributed to management, food and water sources in addition to contacts of cattle with dogs as they acquired the infection by ingesting oocysts. Also, vertical transmission appears to be responsible for the high prevalence of *N. caninum* in cattle **(Anderson *et al.,* 2000).**

The prevalence of *N. caninum* antibodies in cattle was 57.1% in the present study. Similar results reported in Mexico (57.48%) using ELISA (**González *et al.*, 2007**) and in Romania (50%) using ELISA by **Enachescu *et al.*** (**2014**). Also, our results were higher than those reported by several authors in cattle from different regions of the world as in Sudan (10.7%) using ELISA by **Ibrahim *et al.*(2012)** andin Algeria (12.2%) using ELISA by **Miroud *et al.* (2019).** On the other hand, the present results were lower than those reported by several authors in cattle from different regions of the world such as in Brazil (97.2%) using IFAT by **Guedes *et al.* (2008)** and in Argentina (80.9%) with IFAT by **Moré *et al.* (2009).** Because different cut-off points were used in these studies, it is difficult to compare the seroprevalence of antibodies against *N. caninum* in these countries **(Campero *et al.*, 2007).**

The prevalence of *N. caninum* antibodies in buffaloes was 47.2% in the current study. Higher result was recorded by **Gennari, *et al.* (2005)** who found that the prevalence of *N. caninum* was 70.9% in buffaloes using an indirect fluorescent antibody test (IFAT) in Brazil. **Campero *et al.* (2007)** recorded that the prevalence of *N. caninum* was (64%) in Argentina by using IFAT. Also, **Neverauskas *et al.* (2015)** detected high prevalence of *N. caninum* (88.3%) in Australia and **Bărburaș *et al.* (2019)** revealed also high prevalence (68.5%) in northwestern Romania. The variation in these results may be attributed to the difference in the diagnostic technique used, study locality and management. On the other hand, our results were higher than those reported by several authors in buffaloes from different regions of the world as in Italy (34.6%) using IFAT by (**Guarino *et al.*, 2000**) and in the northwest of Iran (19.3%) by ELISA (**Rezvan, *et al.*, 2019**).

This can be explained by the permanent contact between the buffaloes and dogs as contamination source. Furthermore, the lack of attention to the ever increasing stray dog population, the use of dogs for security purpose by most dairy farm owners and poor waste management practice which considered the factors that increases the role of horizontal infection and the prevalence of the neosporosis in both cattle and dogs **(Asmare *et al.*, 2013).**

Buffaloes and cattle are related species that share several parasitic and infectious diseases of economic importance. *N. caninum* is an important cause of abortions in cattle worldwide; therefore, the high seroprevalence observed in buffaloes deserves attention as recorded by **Reichel *et al.* (2013).**

In the present study, the prevalence of *N. caninum* antibodies in dairy cattle and buffaloes according to species were 57.1% and 47.2% respectively by ELISA with no significant differences between two species. Similar results were recorded by **Silva *et al.* (2017)** who stated that the seroprevalence of *N. caninum* was higher in cattle (52%) than buffaloes (39%) in Brazil by IFAT.

In the contrary, **Neverauskas *et al.* (2015)** mentioned that the infection rate of *N. caninum* was higher in water buffaloes (88.3%) than cattle (31.8%) for *N. caninum* antibodies by commercial ELISA and added that *N. caninum* was highly endemic in water buffaloes in Australia. The high prevalence of infection detected in this study might explained due to a high probability of dairy animals being exposed to oocysts throughout their life and the environmental resistance, survival speculation of the *N. caninum* oocysts which being favored by humidity and a moderate temperature **(Dubey *et al.* 2007)**.

In previous studies conducted in different regions of the world several ELISAs were adapted for detection of *Neospora caninum* antibodies in cattle milk samples (**Schares *et al.* 2004 and González-Warleta *et al.* 2011**). Testing of milk samples presents some advantages over testing of blood samples, like easily and lowered costs, noninvasiveness of the method with reduction of some disease transmission by needle and reduction of productions losses caused by stress. So, it can be considered a good diagnostic tool for neosporosis (**Schares *et al.*, 2004**).

Serum antibodies was recorded in an endemic area indicating past or present invasive disease while presence of antibodies in milk indicate present or recent infection which reflect local antigenic stimuli to infection, such antibody detection may help in studies of the endemicity of the disease (**Grundy*et al.*, 1983**).

In the current study, the prevalence of *N. caninum* antibodies in dairy cattle was 5.4% of serum and was 57.1% of milk. Also, the prevalence of *N. caninum* antibodies in dairy buffaloes was 19.4% of serum and was 47.2% of milk. Our result is similar with **Schares *et al.*, (2004)** who found that the milk-based ELISA had a higher sensitivity than the serum-based ELISA. These results explained by **Andrianarivo *et al.* (2001**) who stated that both IgG1 and IgG2 are produced in *N. caninum* infected cattle but at different ratios depending on the time post infection. Shortly after infection IgG1 is produced at much higher rates than IgG2. Since IgG1 is the major IgG subclass present in bovine milk. Lactation stage was identified as a factor that was associated with an increase in the milk ELISA result relative to the serum ELISA result. This can be explained by a decrease in milk yield with increasing time between calving and sampling that leads to higher milk protein and milk IgG concentrations in a later stage of lactation (**Caffin *et al.*, 1993**).On the other hand, **Gerges *et al.* (2018)** detected higher prevalence of *N. caninum* antibodies in serum (29%) than milk samples (10%) in Upper Egypt (El-Fayoum, Giza, Beni -Swief and El-Menia) by ELISA.

In a previous studies performed, it was not observed a correlation between age or seropositivity and neosporosis (**Portocarrero *et al.*, 2015** and **Silva *et al.*, 2015**). Also, we found that the prevalence of *N. caninum* antibodies of dairy cattle by ELISA according to age was 57.1% with age equal or lower than 5 years and was 57.1% with age more than 5 years. This agreed with **Marques *et al.* (2011)** who observed that the prevalence of *N. caninum* in dairy cattle did not proportionally increase with the age of the infected animals. While, the prevalence of *N. caninum* antibodies of dairy buffaloes according to age by ELISA was 58.3% with age equal or lower than 5 years and was 41.7% with age more than 5 years. The age of cattle and buffaloes did not statistically influence the occurrence of *N. caninum* antibodies. This was in agreement with (**Gennari, *et al.,* 2005; Ibrahim *et al.,* 2012** and **Guerra *et al.,* 2019).** In contrary with other authors who noted a significant correlation between the age and number of positive animals (**Guarino *et al.* 2000 and Bărburaș *et al.* 2019**).

The high prevalence of neosporosis in cattle affects the development of the livestock industry and it is also an important infective source for human infection in Delta Egypt (**Ibrahim *et al.*, 2009**). Risk factors associated with *N. caninum* infection in cattle were the presence of more than three dogs in the herd and the disposal of animal waste in the environment

(**Portocarrero *et al.*, 2015**).

In this study, the prevalence of *N. caninum* antibodies of dairy cattle according to housing system by ELISA was 77.8% of farm rearing and was 20% of household rearing which showed very high statistical significant differences between two groups. Despite the fact that, the prevalence of *N. caninum* antibodies among dairy buffaloes by ELISA was 60% of farm rearing and was 38% of household rearing, there were no significant differences of prevalence between two housing groups. In contrary, **Bărburaș *et al.* (2019)** recorded a significantly higher seroprevalence in household buffaloes (74.7%) compared to those originating from farms (35.4%) in the rearing system.

The high infection in farms may be due to continuous exposure to infection with heavy environmental contamination with oocysts shed from the observed stray dogs in the farms with poor management conditions. This agreed with **Dijkstra *et al.* (2002)** who mentioned that mainly farm dogs might be considered a high risk population because the opportunity to eat bovine infected tissues especially bovine placenta. The presence of domestic dogs in the studied farms might facilitate development of a complete lifecycle of the parasite and its persistence in the herds **(Silva *et al.,* 2017)**.
In the present study, the prevalence of *N. caninum* antibodies in cattle milk according to pregnancy status was 18.1% of pregnant cattle and was 66.7% of non-pregnant cattle by ELISA with high significant differences of prevalence of non-pregnant cattle than pregnant ones. While, the prevalence of *N. caninum* antibodies according to pregnancy status of dairy buffaloes in milk by ELISA was 43.8% of pregnant buffaloes and was 50% of non-pregnant buffaloes. Our results agreed with (**Guy *et al.*, 2001 and Trees *et al.*, 2002**) who found that the antibody levels can fluctuate especially during gestation and sometimes fall below the cutoff levels of the commonly used serological assays on both experimentally and naturally *N. caninum*infected cattle**.** Also, Okeoma *et al.* (2004) recorded fluctuatingantibody titres which become seronegative in previouslyseropositive animals have been observed in pregnant cows.

The present study revealed that the aborted buffalo in the 8th month was positive for *N. caninum* antibodies in both serum and milk samples. This is agreed with **Mahajan, *et al.* (2019)** who recorded that abortion due to *N. caninum* was diagnosed in three fetuses (one cattle and two buffaloes) with an average of gestation of 7.5 months. Moreover, the risk of *Neospora*-associated abortion was found to be 2.05 times higher in buffaloes when compared with cows.

Conclusion

The present study confirms the existence of *N. caninum* antibodies in dairy cattle and buffaloes in some localities of Assiut governorate. Milk ELISA represents a valuable tool in screening and monitoring *N. caninum* antibodies more than to be applied on serum samples of dairy cattle and buffaloes.

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