Neosporosis has emerged as a serious disease of cattle and dogs worldwide, causing abortions, neonatal mortalities and massive economic losses in dairy herds. The current study aimed to investigate the burden of *Neospora* (*N.*) *caninum* infection among cows that aborted and to assess knowledge, attitudes, and practices (KAPs) of farmers toward neosporosis in Kafrelsheikh governorate, Egypt. A total of 92 cows that aborted and 25 heifers born from cows that aborted and seropositive for *N. caninum* infection from 15 dairy herds in different districts of Kafrelsheikh governorate were examined serologically against *N. caninum* infection using ELISA. A structured questionnaire was built and distributed to 41 farmers in the study area. The overall seroprevalence of *N. caninum* infection among the examined cows that aborted was 38.04% (35/92). On the other hand, the prevalence of *N. caninum* infection among the 25 examined heifers born from seropositive cows that aborted was 28% (7/25). The KAPs analysis showed that farmers lack the required information on *N. caninum* infection and its consequences on dairy farms. The farmers performed risky practices which are responsible for disease entrance and spread on the farm; buy and keeping animals after abortion as well as their heifers. In addition, risk management practices were widespread in dairy farms such as: free movement of dogs, especially stray ones, in 100% of the farms, dogs were able to access and defecate in cattle feed and drinking water sources and had the chance to eat placentas and abortion materials. The current study points out neosporosis as a neglected abortifacient disease in Egypt, as well as there is a lack of knowledge and risky practices by the local farmers. These findings may be extrapolated to nearby areas and countries of the same cattle husbandry practices, and veterinary services in such countries should consider neosporosis in their surveillance and control programs.

**Keywords:** *Neospora caninum*; cattle; Egypt; knowledge, attitudes and practices; abortion
INTRODUCTION

*Neospora caninum* is an intracellular apicomplexan protozoan parasite recently discovered in Norway in 1984 (Bjerkaas et al., 1984). It infects carnivorines as definitive host and cattle, buffalo, sheep, chickens, and rabbits as intermediate hosts (Dubey and Schares, 2011). Neosporosis in cattle is associated with massive economic losses because of reproductive problems such as abortions, stillbirths, infertility, loss of milk production and premature culling (Bartels et al., 2006, Reichel et al., 2013, Wilson et al., 2016).

Cattle may acquire the infection in two ways, first: horizontally through the ingestion of oocysts that are shed in the feces of infected dogs and this is considered the principal route to the entrance of *N. caninum* infection in dairy herds (Dijkstra et al., 2001a, Gondim et al., 2002). The second way of infection is the transplacental transmission of *N. caninum* where the parasite passes from mother to fetus via the placenta, and this route is considered the most important for sustaining the infection through consecutive pregnancies by congenital infection (Williams et al., 2009, De Aquino Diniz et al., 2019, Japa et al., 2019).

Several risk factors have been proven to be associated with the seroprevalence of neosporosis in dairy herds. The most prominent risk factor is the presence of dogs and their number (Collantes-Fernández et al., 2008, Ribeiro et al., 2019). The presence of rabbits, poultry, foxes, rodents and cats, as well as old age animals was found to be a risk factor for neosporosis in cattle and buffalo (Haddad et al., 2005, Barburas et al., 2019, Olmo, 2019). Moreover, feeding of decayed food has an immunosuppressive effect and was associated as well with a seroprevalence of neosporosis (Bartels et al., 1999). Furthermore, other risk factors for *N. caninum* infection such as handling of cows that aborted and the introduction of new cattle to the herd were also reported (Dario et al., 2013, Llano et al., 2018).

Despite its important role in abortion among cattle herds and the associated economic losses, neosporosis in Egypt has drawn little attention from both farmers and researchers because brucellosis is well known to be the main cause of abortion in dairy farms and this fact obscures the importance of other causes of abortion (El-Diasty et al., 2016, Shalaby et al., 2019). Furthermore, the diagnosis of *N. caninum* infection is hard and expensive (Ahmed et al., 2017). On the other hand, animal husbandry systems in Egypt, where most farms located near to rural areas of villages sustain the favorable environment for *N. caninum*, due to the presence of dogs with high numbers roaming freely around and enter the cattle farms and the lack of veterinary attention. The scarce researches on *N. caninum* in Egypt showed that it is endemic at moderate levels; 68%, in buffaloes (Dubey et al., 1998), 3.6% in camels (Hilali et al., 1998), 20.43% in cattle, 1.85% in rabbits and 7.92% in human in Northern Egypt (Ibrahim et al., 2009), 14.75% in chickens in Kafrelsheikh (Ibrahim et al., 2013), and 18.9% in cattle in southern Egypt (Fereig et al., 2016).

Antibodies in the serum of infected animal are very important for diagnosis of *N. caninum* infection, which could be detected by different serological tests, including: immunoblotting (IB), direct agglutination test (DAT), indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assays (ELISAs) (Söndgen et al., 2001, Moraveji et al., 2012, Moore et al., 2014, Hamidinejat et al., 2015). Serological surveys for capturing sero-prevalence data are much required for control neosporosis (Fereig et al., 2016). Therefore, a sero-survey for *N. caninum* infection among cows that aborted in dairy herds may highlight the importance of the neglected role of such protozoan for abortion in Egyptian farms and withdraw the attention towards different causes of abortion other than brucellosis.

The objectives of the current study were to determine the seroprevalence of *N. caninum* among cows that aborted in Kafrelsheikh governorate. Furthermore, for the first time up to our knowledge, to determine, the knowledge, attitude, and practices of the farmers towards *N. caninum* infection in dairy herds which may help in the spread of the disease.

MATERIAL AND METHOD

Study Area

Kafrelsheikh is an agricultural governorate which lies in the Northern part of Egypt. It is bordered in the North by the Mediterranean Sea, in the West by Rosetta branch of the River Nile, that stretch by 85 km till its mouth in the Mediterranean Sea, in the East, by Dakahlia governorate, and in the South by Gharbia governorate. Total area estimated as (3466.69 Km2) while, the total population estimated as (3,386,270). The governorate has 10 districts: Kafrelsheikh, El Hammool, Baltim, Biyala, Desouk, Fuwwah, Metobues, Qallin, El Reyad, and Sidi Salem. The map of the study area is illustrated in Figure 1 and was created using Quantum GIS (Quantum GIS Development Team 2017).
Animals and sampling

Fifteen cattle herds in four districts of the Kafrelsheikh governorate (Figure 1) were included in this study with a total number of 935 cows. These herds were suffering from the repeated occurrence of endemic and sporadic abortion in the late gestation period, of unidentified etiology. A total of 92 cows that aborted in these herds, and 25 heifers born from cows that aborted and seropositive for *N. caninum* infection were blood-sampled and examined in this study. All Kafrelsheikh University and international guidelines for the care and use of animals in scientific research according to Vasbinder and Locke (2016) were followed-up. The collected plain blood samples (n= 117) were immediately transported to the diagnostic laboratory. Serum was separated after centrifugation at 3000 rpm for 10 minutes and was kept at -20 °C till be used in ELISA test.

Data collection

A structured questionnaire was used for collecting data on the knowledge, attitudes, and practices of cattle farmers in the study area regarding neosporosis. Knowledge of the farmers regarding neosporosis and source of infection was assessed through both direct and indirect closed questions on the owner’s awareness of the diseases. Other questions to identify farmers’ attitudes and practices related to *N. caninum* infection and transmission were included in the questionnaire. The questions focused on what is the role of dogs in the transmission of the disease, are the farmers protecting water sources and feedstuff from contamination with dog feces, how could they handle cows that aborted and the abortion materials, what is the behavior of cows toward the abortion materials, and finally how do the dogs, particularly stray dogs, behave upon entrance to the farms. The questionnaire was developed in English and the main author of the work was responsible to deliver it to farmers, explain the questionnaire aims and contents in Arabic to the farmers, fill in the questionnaire, stored collected data on Microsoft excel 2007 and carried out the statistical calculation on excel using its built-in functions.

Serological examination

All sera were tested for antibodies against *N. caninum* by using a commercially available ELISA kit (IDEXX Laboratories, United States) coated with *N. caninum* antigen. 10 µl of each serum sample as well as the provided positive and negative controls was diluted in 90 µl of sample diluent. The microtiter plate was incubated at 37 °C for 60 minutes. After three washing steps, 100 µl of the conjugate was added to each well and incubated under the same conditions. The washing steps were repeated and 100 µl of TMB substrate was added into each well and incubated at
20°C for 15 minutes. The reaction was stopped by adding 100 µl stopping solution. The microtiter plate was read at a wavelength of 450nm. The results were interpreted according to the producer equation:

\[
S/P\ % = 100 \times \frac{\text{Sample OD} - \text{Negative control OD}}{\text{Positive control OD} - \text{Negative control OD}}
\]

Where OD is the optical density of samples.

Sample of S/P % ≥40% is considered a positive sample to \textit{N. caninum} infection.

**RESULTS**

The overall seroprevalence of \textit{N. caninum} in the examined cows that aborted was 38.04% (35/92), the distribution of seropositive cows that aborted by the district is shown in (Table 1). On the other hand, the prevalence of \textit{N. caninum} among the 25 examined heifers born from seropositive cows that aborted, was 28% (7/25) (Table 2).

**Results of KAPs assessment**

The results of the questionnaire survey to assess the farmers’ KAPs in Egypt towards neosporosis are shown in (table 3). All of the farmers who participated in the study (N= 41) do not know about \textit{N. caninum} infection and its role in the abortion of their cows. Furthermore, they confirmed that they do not know the role of dogs in disease transmission to their animals.

| District       | Number of examined farms | Number of cows that aborted | Number of seropositive animals % |
|----------------|--------------------------|-----------------------------|---------------------------------|
| Kafrelsheikh   | 5                        | 37                          | 14 (37.8%)                      |
| Biyala         | 4                        | 23                          | 11 (47.8%)                      |
| Desouk         | 3                        | 15                          | 7 (46.7%)                       |
| Sidi salem     | 3                        | 17                          | 3 (17.6%)                       |
| Total          | 15                       | 92                          | 35 (38.04%)                     |

| District       | Number of heifers born from seropositive aborted dams | Number of seropositive heifers % |
|----------------|--------------------------------------------------------|---------------------------------|
| Kafrelsheikh   | 17                                                     | 5 (29.4%)                       |
| Biyala         | 7                                                      | 2 (28.6%)                       |
| Total          | 25                                                     | 7 (28.0%)                       |

**Table 3: Knowledge, attitude and practices of cattle farmers’ towards \textit{N. caninum} at Kafrelsheikh governorate, Egypt.**

| Topics                                      | Yes Percentage |
|---------------------------------------------|----------------|
| Quarantine for newly purchased animals on the farm | 2.4%           |
| Asking of the history of abortion of new abortion cases | 0.0%           |
| Buy cows previously known to have abortion, for breeding | 7.3%           |
| Source of drinking water in your farm        |                |
| Ponds and lakes                             | 24.4%          |
| Tap water supply                            | 75.6%          |
| Type of reproductive service                |                |
| AI                                          | 9.7%           |
| Natural mating                              | 48.8%          |
| Both of them                                | 41.5%          |
| History of abortion last year               |                |
| Prevalence of abortion on the farm          | 100%           |
| < 10%                                       | 80.5%          |
| > 10%                                       | 19.5%          |
| Identification of the causative agents of abortion | 7.3%          |
| Vaccination against infectious abortifacient agents like RVF, Brucellosis….etc | 0.0%          |
| Percentage of cows with repeated abortion on the farm | 58.5%          |
| Knowledge of Neospora caninum               | 0.0%           |
| Reduction in milk of animals suffered from abortion | 9.8%          |
| Decreasing in the fertility in animals had abortion | 58.5%          |
| Keep heifers born from previously had abortion and \textit{N. caninum} seropositive cows for breeding | 100.0%         |
| Cows eating placentas in farms              | 19.5%          |
| Cows licking in abortion uterine discharge on the farm | 92.7%          |
Abortion occurs mainly in

| Category   | Percentage |
|------------|------------|
| Heifers    | 2.4%       |
| Cows       | 85.4%      |
| Both       | 12.2%      |

Dealing with cows that aborted

| Action                  | Percentage |
|-------------------------|------------|
| Keep on the farm         | 87.8%      |
| Sell or slaughter        | 12.2%      |

Handling of aborted feti, materials and placentas on the farm

| Action                  | Percentage |
|-------------------------|------------|
| Throw them outside the farm | 73.2%      |
| Present/leave them to dogs on the farm | 22.0%      |
| Burial                  | 2.4%       |
| Burning                 | 2.4%       |

Noticing disease complication in calves, like nervous manifestation and paralysis

| Description                                                   | Percentage |
|---------------------------------------------------------------|------------|
| Normally use and sell it                                     | 92.7%      |
| Get rid of it                                                 | 7.3%       |

Provide suckling calves with milk from dams

| Action                  | Percentage |
|-------------------------|------------|
| 100%                    |            |

Entrance of dogs to the farm

| Description                                                   | Percentage |
|---------------------------------------------------------------|------------|
| Freely rooming on the farm                                   | 80.5%      |
| Short visits                                                 | 19.5%      |

Type of dogs entered the farm

| Description                                                   | Percentage |
|---------------------------------------------------------------|------------|
| Owned dogs                                                    | 68.3%      |
| Stray dogs                                                    | 31.7%      |

Age of the dogs entered the farm

| Description                                                   | Percentage |
|---------------------------------------------------------------|------------|
| Adult dogs only                                               | 90.3%      |
| Both of Bubbies and adult dogs                                | 9.7%       |

Observing of bitches giving birth on the farm

| Description                                                   | Percentage |
|---------------------------------------------------------------|------------|
| 19.5%                                                         |            |

Observing of dogs eating abortion materials and placenta on the farm

| Description                                                   | Percentage |
|---------------------------------------------------------------|------------|
| 100%                                                         |            |

Dogs defecate on silage and green fodders

| Description                                                   | Percentage |
|---------------------------------------------------------------|------------|
| 92.7%                                                         |            |

Dogs defecate on drinking water supply

| Description                                                   | Percentage |
|---------------------------------------------------------------|------------|
| 29.3%                                                         |            |

Putting the remnant fodder to heifers on the farm

| Description                                                   | Percentage |
|---------------------------------------------------------------|------------|
| 48.8%                                                         |            |

Knowledge of the role of dogs for transmission of infectious diseases to his cows

| Description                                                   | Percentage |
|---------------------------------------------------------------|------------|
| 0.0%                                                         |            |

Noticing disease complication in these dogs, like nervous manifestation and paralysis

| Description                                                   | Percentage |
|---------------------------------------------------------------|------------|
| 0.0%                                                         |            |

All farmers declared that their cows suffered from abortion in the last year before the current study. The prevalence of abortion in 80.5% of the herds was < 10% and 58.5% of farmers experienced repeated abortions among their animals. All farmers kept heifer born from animals with a previous abortion for breeding and they do not ask for the history of abortion for newly purchased animals, but 7.3% only of the farmers will still buy cows that aborted if they knew their abortion history. A total of 92.6% of the farmers noticed that cows that abort lick their abortion discharges and 19.5% of the farmers confirmed that the cows eat their placentas after abortion. The decrease in the fertility and milk production after abortion was recorded by 58.5% and 9.8% of the farmers, respectively. Abortion was recorded mainly among adult cows in 58.4% of the farmers’ answers. None of the farmers vaccinated against the causative agents of abortion and only 7.4% of them identified the microbial causative agent of abortion in their herds. The cattle that abort are kept on the farm by 87.8% of the farmers and 95% and 100% of them use and/or sell the milk of these animals and offered this milk to suckling calves, respectively. On the other hand, 22.0% of the farmers presented or leave the abortion materials to dogs.

Dogs are owned by 68.3% of the farmers and kept on the farm/herd. On the other hand, 100% of farmers see the dogs in their herds - mainly adult dogs- either owned or stray despite the presence of fences in 75% of these farms to prevent external dog entrance. Around 92.6%, 92.2% and 19.2% of the farmers noticed that dogs defecate on feedstuff and in water supply for cattle, and giving birth inside the farm, respectively.
spectively. The decayed feedstuff is being provided to heifers as reported by 48.8% of the farmers.

**DISCUSSION**

*N caninum* infection is a major cause of abortion in dairy herds and so it hinders the attempts to progress livestock productivity in the main source of income to low-middle income countries such as Egypt (Perry and Grace, 2009, Reichel et al., 2013, Semango et al., 2019). There is a little attention given to *N. caninum* in the problem of abortion in cattle in Egypt. This is up to our knowledge the first sero-epidemiological report of *N. caninum* infection among cows that aborted in Egypt’s dairy cattle herds and the first KAPs analysis of the farmers towards such infection.

The results of the current study declared that *N. caninum* infection is a neglected widely spread problem in dairy herds of Kafrelsheikh governorate, Egypt. The existence of all suitable environmental and management aspects of disease spread as obtained from the answers of the farmers to the questionnaire on the farmers’ KAPs towards *Neospora* infection is the reason for this high prevalence obtained.

Environmental aspects of disease spread in Kafrelsheikh governorate represented in its topography as an agricultural governorate with a lot of rural areas in and around the farms and herds which encourage the presence of dogs with high numbers. In the current study, farmers confirmed the usual presence of dogs, including stray dogs roaming-in their farms, and declared that dogs can defecate in water and feedstuff which represents a major risk for infection because *N. caninum* oocysts release with feces of dogs (Lindsay et al., 1999, Dijkstra et al., 2001b, De Souza et al., 2002). The oocyst-contaminated ration and water are the principal routes of horizontal transmission of *N. caninum* and they are responsible for the entrance of infection to neosporosis-free dairy herds (Dubey et al., 2007).

The lack in knowledge of the farmers about the disease, its sources of infection and routes of transmission are responsible for the risk practices which they perform and increase the chance of neosporosis spread. Keeping cows that abort, buying animals that had aborted, breeding of heifers born from cows that aborted and offering the abortion materials to dogs which completes the cycle of *N. caninum* infection are management practices increase the risk of horizontal and vertical transmissions of *N. caninum* in dairy herds. Such findings are compatible with Dijkstra et al. (2001a), Gondim et al. (2002), Williams et al. (2009), Trees and Williams. (2005), who confirmed that farms with bad hygienic management with cows that aborted and their related materials as (abortion placenta, and aborted fetuses and uterine discharges) increase probability of *Neospora* infection because these materials constitute the sources of infection especially for dogs which eat these materials. Also, keep the cows that aborted in the farms increase the probability of calves being seropositive due to transplacental infection with *N. caninum*. Another risky management factor for neosporosis spread in dairy herds is the ignorance of the veterinarians to identify the causative agents responsible for abortion in the farms either by bacteriological or serological examinations. Farms without policies of regular serological testing and culling of cows that aborted and vaccination toward diseases causing an abortion, increase the probability of *N. caninum* infection by increasing the number of seropositive cows in these farms (Hall et al., 2005, Weston et al., 2012, Aguado-Martínez et al., 2019).

Semango et al. (2019) found a positive association between within-herd *N. caninum* seroprevalence and abortion rates among cattle in Tanzania. The resulted high seroprevalence of *N. caninum* infection among cows that aborted indicates the neglected role of *N. caninum* infection as the causative agent of abortion in dairy herds in Egypt. Previous studies showed lower seroprevalence values in cattle such as Ibrahim et al. (2009) and Fereig et al. (2016) who reported seroprevalences of 20.43% and 18.9% in northern and southern Egypt, respectively. Similarly, lower prevalences were reported in other countries such as in Tanzania by Semango et al. (2019), who reported a prevalence of 21.5% and such as in other African countries such as Ghalmi et al. (2012), who reported prevalence of 10.7% and 19.6% in cows. The higher seroprevalence found in the current study than that reported by others is because the sampling in the current study was only from cows suffered from abortion. Other factors which influence the prevalence of neosporosis in cattle include the temporal and geographical distribution of different studies, the type of the used serological test, cattle husbandry practices and immune response of animals upon exposure to infection (Innes et al., 2002, Santolaria et al., 2011, Bartley et al., 2013).

The vertical mode of the disease transmission contributed to the maintenance of *N. caninum* infection
in the herd over several following bovine generations (Ortega-Mora et al., 2007). The present study showed that the high prevalence of *N. caninum* among heifers born from cows that aborted and *N. caninum* seropositive was (28%).

Significantly, some previous surveys also recorded a very high rate of congenital transmission in seropositive cows as 94% (French et al., 1999), 36.8% (Vianna et al., 2008), 67.53% (Andreotti et al., 2010), and 55.5% as reported by Lagomarsino et al. (2019).

The obtained results in the current study could be widely upscaled to other neighboring countries with similar animal production systems and lack of information on *N. caninum* infection in dairy herds.

**CONCLUSIONS**

This study results indicated a high prevalence of *N. caninum* infection among cows that aborted in dairy herds in Egypt. Despite this high prevalence, neosporosis is still a neglected problem among farmers and incorrect farm management practices widely exists which contributed to the spread of *N. caninum* infection entire dairy herd. Therefore, there is a must supply for the data about *N. caninum* infection to increase knowledge for the managers of farms and herd in Egypt about this parasite. Finally, obtained results emphasize the need to implement prevention programs of *N. caninum* infection and strategies for the culling of seropositive cows in dairy herds in Egypt.

**CONFLICT OF INTEREST**

The authors declared that there is no conflict of interest.

**REFERENCES**

Aguado-Martínez A, Basto AP, Tanaka S, Ryser LT, Nunes TP, Ortega-Mora LM, Arranz-Sols D, Leitão A, Hemphill A (2019) Immunization with a cocktail of antigens fused with OprF reduces *Neospora caninum* vertical transmission and postnatal mortality in mice. Vaccine 37: 473-483.

Ahmed NE, Al-Akabway LM, Ramadan MY, Abd El-Gawad SM, Mousa TM, Shafik SM, El-Diasty MM, Ahmed HA, Sayour AE, El Hofy FI, Tahoun AB, Shafik SM (2011) Neosporosis in animals - The last five years. Vet Parasitol 180: 279-292.

Andreotti R, Barros CJ, Pereira AR, Oshiro LM, Cunha RC, Neto LFF (2010) Association between seropositivity for *Neospora caninum* and reproductive performance of beef heifers in the Pantanal of Mato Grosso do Sul, Brazil. Rev Bras Parasitol Vet 19: 119-123.

Bărburaş D, Györke A, Blaga R, Bărburaş R, Kalmár Z, Vişan S, Mircean C, Secăreanu MMA (2017) Serological and PCR-sequencing assays for diagnosis of *Toxoplasma gondii* and *Neospora caninum* infecting camels in Egypt. BVM 33: 200-210.

Bartels CJ, Wouda W, Schukken YH (1999) Risk factors for *Neospora caninum*-associated abortion storms in dairy herds in The Netherlands (1995 to 1997). Theriogenology 52: 247-257.

Bartels CJ, Van Schaijk G, Veldhuisen JP, Van den Borne BH, Wouda W, Dijkstra T (2006) Effect of *Neospora caninum*-serostatus on culling, reproductive performance and milk production in Dutch dairy herds with and without a history of *Neospora caninum* associated abortion epidemics. Prev Vet Med 77: 186-198.

Barley PM, Katzer F, Rocchi MS, Maley SW, Benavides J, Nath M, Pang Y, Cantón G, Thomson J, Chianini F, Innes EA (2013) Development of maternal and foetal immune responses in cattle following experimental challenge with *Neospora caninum* at day 210 of gestation. Vet. Res. 44: 91.

Bjerkaas I, Mohn SF, Prestus J. (1984) Unidentified cyst-forming sporeforming causing encephalomyelitis and myositis in dogs. Z Parasitenkd 70: 271-274.

Collantes-Fernández E, Gómez-Bautista M, Miró G, Alvarez-Garcia G, Pereira-Bueno J, Frisuecos J, Ortega-Mora LM (2008) Seroprevalence and risk factors associated with *Neospora caninum* infection in different dog populations in Spain. Vet Parasitol 152: 148-151.

Dario Cedeño Q, Bibiana Benavides B (2013) Seroprevalence and risk factors associated to *Neospora caninum* in dairy cattle herds in the municipality of Pasto, Colombia. Rev MVZ Córdoba 18: 3311-3316.

De Aquino Diniz LV, Minuti AF, de Souza Lima Nino B, Costa LR, Bosculeo MRM, de Almeida BF, Garcia JL, de Barros LD (2019) Vertical transmission of *Neospora caninum* in bovine fetuses from a slaughterhouse in Brazil. Trop Anim Health Prod 51:175-1755.

De Souza SL, Guimaraes JS, Ferreira F, Dubey JP, Gennari SM (2002) Prevalence of *Neospora caninum* antibodies in dogs from dairy cattle farms in Paraná, Brazil. J Parasitol 88: 408-409.

Dijkstra T, Barkema HW, Eyserk M, Wouda W (2001a) Evidence of post-natal transmission of *Neospora caninum* in Dutch dairy herds. Int J Parasitol 31: 209-215.

Dijkstra T, Eyserk M, Schares G, Conraths FJ, Wouda W, Barkema HW (2001b) Dogs shed *Neospora caninum* oocysts after ingestion of naturally infected bovine placenta but not after ingestion of colostrum spiked with *Neospora caninum* tachyzoites. Int J Parasitol 31: 747-752.

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 Romania: what is the importance for public health? Parasitol Res. 118:2695-703.

Dubey JP, Schares G, Ortega-Mora LM (2007) Epidemiology and control of neosporosis and *Neospora caninum*. Clin Microbiol Rev 20: 323-367.

Dubey JP, Schares G (2011) Neosporosis in animals - The last five years. Vet Parasitol 180: 90-108.

El-Diasty MM, Ahmed HA, Sayour AE, El Hofy FI, Tahoun AB, Shafik SM (2016) Seroprevalence of *Brucella* spp. in cattle, Molecular Characterization in Milk, and the Analysis of Associated Risk Factors with Seroprevalence in Humans, Egypt. Vector Borne Zoonotic Dis 16:758-764.

Fereig RM, AbouLaila MR, Mohamed SG, Mahmoud HYAH, Ali AO, Ali AF, Hilali M, Zaid A, Mohamed AEA, Nishikawa Y (2016) Serological detection and epidemiology of *Neospora caninum* and *Cryptosporidium parvum* antibodies in cattle in southern Egypt. Acta Trop 162: 206-211.

French NP, Davison HC, Clancy D, Trees AJ (1999) Mathematical models of *Neospora caninum* infection in dairy cattle, transmission and options of control. Int J Parasitol 29: 691-704.

Ghalim F, China B, Ghalim A, Hammoumouch D, Lasson B (2012) Study of the risk factors associated with *Neospora caninum* seroprevalence in Algerian cattle populations. Res Vet Sci 93: 655-661.

Gondim LF, Gao L, McAllister MM (2002) Improved production of *Neospora caninum* oocysts, cyclical oral transmission between dogs
and cattle, and in vitro isolation from oocysts. J Parasitol 88: 1159-63.
Haddad JP, Dohoo IR, VanLeeuwen JA (2005). A review of Neospora caninum in dairy and beef cattle—a Canadian perspective. Can Vet J 46: 230-243.
Hall CA, Reichel MP, Ellis JT (2005) Neospora abortions in dairy cattle: diagnosis, mode of transmission and control. Vet Parasitol 128: 231-241.
Hamidnejat H, Seifi Abad Shapouri MR, Namavari MM, Shayan P, Kefayat M (2015) Development of an Indirect ELISA using different fragments of recombinant Ncgra7 for detection of Neospora caninum infection in cattle and water buffalo. Iran J Parasitol 10: 69-77.
Hilali M, Romand S, Thulliez P, Kwok OC, Dubej JP (1998) Prevalence of Neospora caninum and Toxoplasma gondii antibodies in sera from camels from Egypt. Vet. Parasitol 75:269-271.
Ibrahim HM (2013) Seroprevalence of Neospora caninum antibodies and Toxoplasma gondii in camels from Egypt. J. Immuno 20:29-37.
Innes EA, Andrianarivo AG, Björkman C, Williams DJ, Conrad PA (2002) Immune responses to Neospora caninum and prospects for vaccination. Trends Parasitol 18: 497-504.
Japa O, Nuanmee K, Prakhammin K, Flynn RJ (2019) Prevalence of vertically transmitted Neospora caninum amongst beef cattle in Phayao. Thailand. Parasitol Int 70: 98-101.
Lagomarsino H, Sciolli A, Rodríguez A, Armendano J, Fiorani F, Bence Japa O, Nuangmek A, Prakhammin K, Flynn RJ (1998) Prevalence of Neospora caninum and Toxoplasma gondii antibodies in northern Egypt. Am J Trop Med Hyg 80: 263 - 267.
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: 29-37.
Innes EA, Andrianarivo AG, Bjorkman C, Williams DJ, Conrad PA (2002) Immune responses to Neospora caninum and prospects for vaccination. Trends Parasitol 18: 497-504.
Japa O, Nuanmee K, Prakhammin K, Flynn RJ (2019) Prevalence of vertically transmitted Neospora caninum amongst beef cattle in Phayao. Thailand. Parasitol Int 70: 98-101.
Lagomarsino H, Sciolli A, Rodriguez A, Armendano J, Fiorani F, Bence J, Garcia J, Hecker Y, Gual I, Cantón G, Odeón A, Campero C, Moore D (2019) Controlling Endemic Neospora caninum infection in a Dairy Herd From Argentina. Front Vet Sci 6: 446.
Llano HA, Guimarães MS, Soares RM, Gima Pola, Caetanoa S (2018) Seroprevalence and risk factors for Neospora caninum infection in cattle from the eastern Antioquia, Colombia. Vet Anim Sci 6: 69-74.
Lindsay DS, Dubej JP, Duncan RB (1999) Confirmation that the dog is a definitive host for Neospora caninum. Vet Parasitol 82: 327-333.
Moraveji M, Hosseini A, Moghaddar N, Namavari MM, Eskandari MH (2012) Development of latex agglutination test with recombinant NcSAG1 for the rapid detection of antibodies to Neospora caninum in cattle. Vet Parasitol 189: 211-217.
Moore DP, Konrad JL, San Martino S, Reichel MP, Cano DB, Mendez S, Spáth EJ, Odeón AC, Crudeli G, Campero CM (2014) Neospora caninum serostatus is affected by age and species variables in cohabiting water buffaloes and beef cattle. Vet Parasitol 203: 259-263.
Olmo L, Reichel MP, Nampanya S, Khounsy S, Wahl LC, Clark BA, Thomson PC, Windsor PA, Bush RD (2019) Risk factors for Neospora caninum, bovine viral diarrhea virus and Leptospira interrogans serovar Hardjo infection in smallholder cattle and buffalo in Lao PDR. PloS One 14: 1-25.
Ortega-Mora LM, Gottstein MB, Conraths FJ, Buxton D (2007) Protozoal abortion in farm ruminants: Guidelines for Diagnosis and Control. 1st ed, Athenaeum Press, Oxfordshire, UK: pp 302.
Perry B, Grace D (2009) The impacts of livestock diseases and their control on growth and development processes that are pro-poor. Philos Trans R Soc Lond B Biol Sci 364: 2643-2655.
Reichel MP, Alejandro Ayangui-Alcêrrea M, Gondim LF, Ellis JT (2013) What is the global economic impact of Neospora caninum in cattle - the billion dollar question. Int J Parasitol 43: 133-142.
Ribeiro CM, Soares JR, Mendes RG, de Santos Bastos PA, Katagiri S, Zavilenski RB, de Abreu HFP, Afreixo V (2019) Meta-analysis of the prevalence and risk factors associated with bovine neosporosis. Trop Anim Health Prod 51: 1783-1800.
Sanotoria L, Almeria S, Martinez-Bello D, Nogareda C, Mezo M, Gonzalez-Larreta M, Castro-Hermida JA, Pabón M, Yáñez JL, López-Gatius F (2011) Different humoral mechanisms against Neospora caninum infection in pure breed and crossbreed beef/dairy cattle pregnancies. Vet. Parasitol 178: 70-76.
Semango G, Hamilton CM, Krepel K, Fikoina T, Lankester F, Allan KJ, Thomas KM, Claxton JR, Innes EA, Swai ES, Buza J, Cleaveiand S, de Glanville WA (2019) The Sero-epidemiology of Neospora caninum in Cattle in Northern Tanzania. Front Vet Sci 6:327.
Shalaby Y, Mousa W, Elbaz HT, Sherif HR, Nayel M, Elsify A, Zagha- wa A, Salama A, Elmenshaway A, Hadad GA, Shaker A, Eldosoky I, Heavin H (2019) Serological and Molecular Diagnosis of Bovine Brucellosis in Menoufia Province. JCVR 1:115-126.
Söndgen P, Peters M, Bärwald A, Wurm R, Holling F, Conraths FJ, Schøn G (2001) Bovine neosporosis: immunoblot improves serological. Vet. Parasitol 102: 279-290.
Trees AJ, Williams DJ (2005) Endogenous and exogenous transplacental infection in Neospora caninum and Toxoplasma gondii. Trends Parasitol 21: 558-561.
Vasbinder MA, Locke P (2016) Introduction: global laws, regulations, and standards for animals in research. ILAR journal 57(3): 261-265.
Vianna LC, Sartor IF, Pituco EM, Okada LH, Camargo CN, Krona SN (2008) Incidence and transplacental transmission of Neospora caninum in primiparous females from Bosindicus slaughtered in Presidente Prudente, SãoPaulo, Brazil. Semina. Ciências Agrárias, Londrina 29:387-392.
Weston DJ, Heuer C, Williamson NB (2012) Efficacy of a Neospora caninum killed tachyzoite vaccine in preventing abortion and vertical transmission in dairy cattle. Prev Vet Med 103: 136-144.
Williams DJ, Hartley CS, Björkman C, Trees AJ (2009) Endogenous and exogenous transplacental transmission of Neospora caninum - how the route of transmission impacts on epidemiology and control of disease. Parasitology 136: 1895-1900.
Wilson DJ, Orsel K, Waddington J, Rajeev M, Sweeny AR, Joseph T, Grigg ME, Raverty SA (2016) Neospora caninum is the leading cause of bovine fetal loss in British Columbia, Canada. Vet Parasitol 218: 46-51.