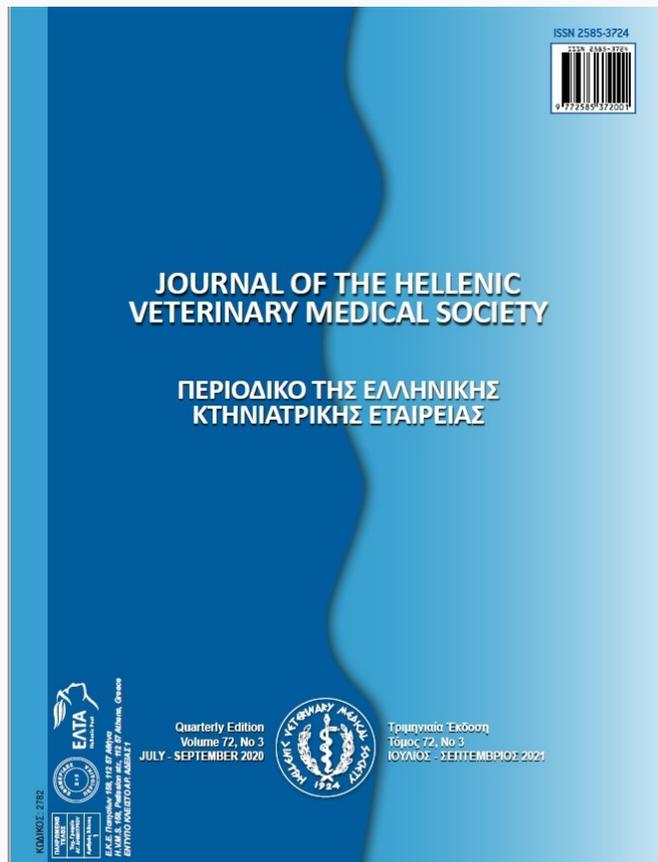


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Neosporosis: a neglected abortifacient disease in Egypt, seroprevalence and farmers' knowledge, attitudes and practices

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ABSTRACT: 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 cause of abortion among cattle in Kafrelsheikh governorate, 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

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INTRODUCTION

Neospora caninum is an intracellular apicomplexan protozoan parasite recently discovered in Norway in 1984 (Bjerkas et al., 1984). It infects carnivores 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 (Darío 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 sus-

tain 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 METHODS

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 Hamool, Baltim, Biyala, Desouk, Fuwwah, Metoubes, 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).

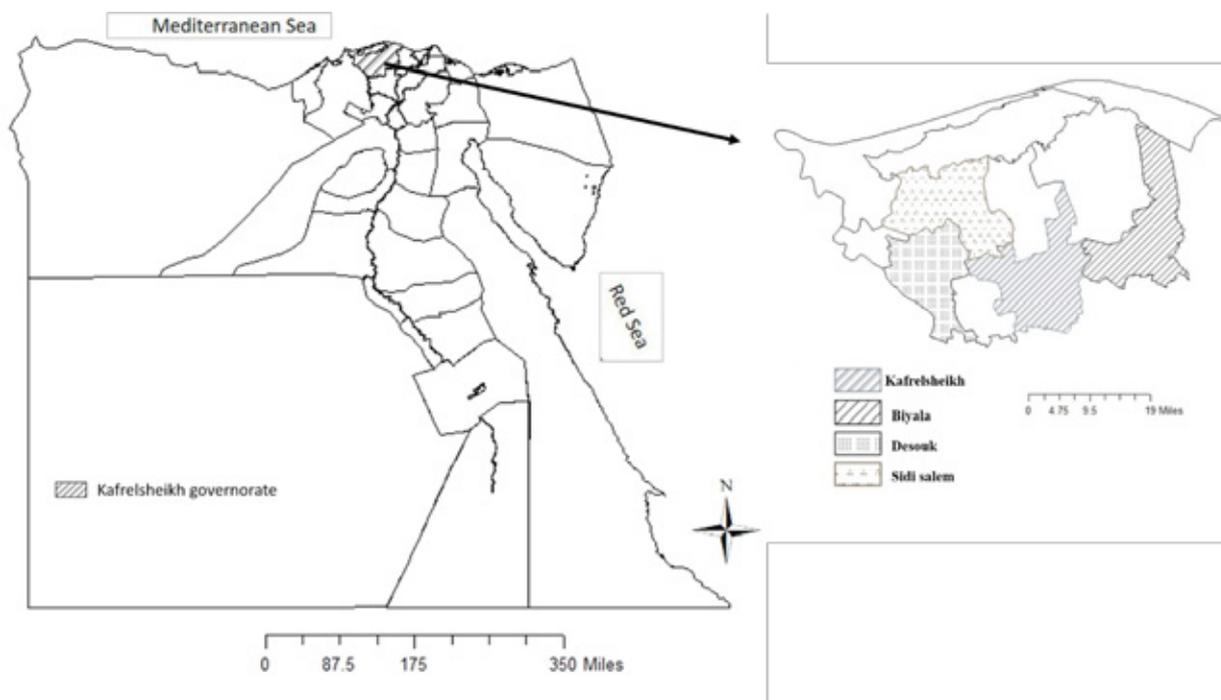


Figure 1: A choropleth map of Egypt showing the administrative boundaries of Kafrelsheikh governorate on the left side and the study area districts at this governorate on the right side

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. cani-*

num 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:

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

Where OD is the optical density of samples

Sample of S/P % \geq 40% is considered a positive sample to *N. caninum* infection.

RESULTS

The overall seroprevalence of *N. caninum* in the examined cows that aborted was 38.04% (35/92), the

Table 1: Seroprevalence of *N. caninum* infection among cows that aborted at Kafrelsheikh governorate, Egypt.

District	Number of 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) %

distribution of seropositive cows that aborted by the district is shown in (Table 1). On the other hand, the prevalence of *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 *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.

Table 2: *N. caninum* infection seroprevalence among heifers born from seropositive dams at Kafrelsheikh governorate districts, Egypt.

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 *N. caninum* at Kafrelsheikh governorate, Egypt.

Topics	Yes
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	100%
Prevalence of abortion on the farm	
< 10%	80.5%
> 10%	19.5%
Identification of the causative agents of abortion	7.3%
Vaccination against infectious abortifacient agents like RVE, Brucellosis....etc	0.0%
Percentage of cows with repeated abortion on the farm	58.5%
Knowledge of <i>Neospora caninum</i>	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 <i>N. caninum</i> 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	
Heifers	2.4%
Cows	85.4%
Both	12.2%
Dealing with cows that aborted	
Keep on the farm	87.8%
Sell or slaughter	12.2%
Handling of aborted feti, materials and placentas on the farm	
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	
	26.8 %
Handling of milk of cows that suffered from abortion	
Normally use and sell it	92.7%
Get rid of it	7.3%
Provide suckling calves with milk from dams	
	100%
Entrance of dogs to the farm	
	100%
Fences around farms to prevent stray dogs' entrance	
	75.6%
Dogs behavior on the farm	
Freely rooming on the farm	80.5%
Short visits	19.5%
Type of dogs entered the farm	
Owned dogs	68.3%
Stray dogs	31.7%
Age of the dogs entered the farm	
Adult dogs only	90.3%
Both of Bubbies and adult dogs	9.7%
Observing of bitches giving birth on the farm	
	19.5%
Observing of dogs eating abortion materials and placenta on the farm	
	100%
Dogs defecate on silage and green fodders	
	92.7%
Dogs defecate on drinking water supply	
	29.3%
Putting the remnant fodder to heifers on the farm	
	48.8%
Knowledge of the role of dogs for transmission of infectious diseases to his cows	
	0.0%
Noticing disease complication in these dogs, like nervous manifestation and paralysis	
	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 farm-

ers 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, re-

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 rooming-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 Ghalimi 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.

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