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SURVEILLANCE OF NEOSPOROSIS AMONG DIFFERENT SPECIES OF ANIMALS IN DIFFERENT LOCALITIES IN EGYPT

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ABSTRACT

A total of 350 blood samples were collected from 104 cows, 169 ewes and 77 buffalo dams in 6 governorates in Egypt over a full one-year period (2016). Sera were tested for detection of *Neospora caninum* antibodies using commercial ELISA kit. From the 350 animals, 124 had a previous record of abortion. Antibodies to *Neospora caninum* were detected in 79 out of 350 serum samples among all animals in 6 governorates in Egypt with a percentage of 22.57%. Out of those 79 animals, seropositive cows were 34 (43.04%); ewes were 32 (40.51%) and buffaloes were 13 (16.46%). The Seroprevalence was 50, 36.76 and 13.33 % in aborted cows, ewes and buffaloes respectively. The prevalence of *N. caninum* antibodies was higher in the aborted females than in normal ones as it was 24.19 % in aborted females compared to 21.68 % in non-aborted females. . Regarding the geographical prevalence, the high incidence was (35.71%) in Beni-sweif Governorate followed by (25%) in Aswan; (20%) in Menia and Sohag; (16%) in Giza and lowest (10%) in Fayoum.

Further studies to clarify the impact of neosporosis on the animal industry, losses due to clinical neosporosis in livestock in Egypt and application of control strategies for such disease in dairy farms is recommended.

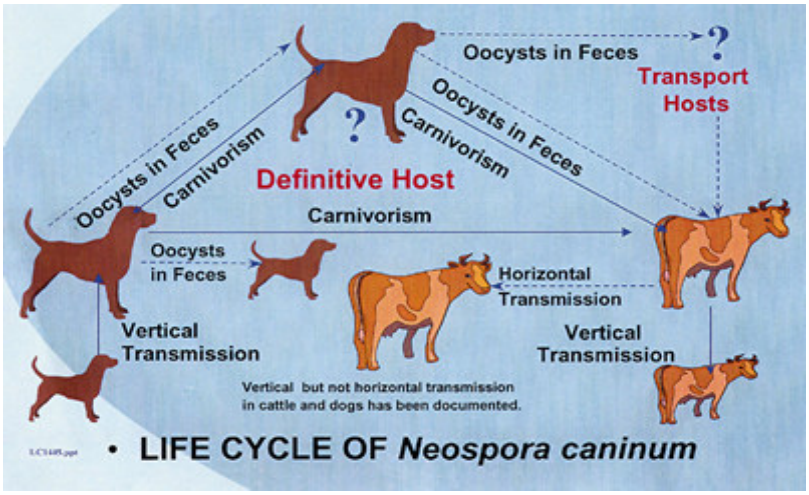
Key words:

Neospora caninum, ELISA kit, control strategies.

INTRODUCTION

Neospora caninum is an apicomplexan protozoan that was first recognized in dogs in Norway **(Bjerkas *et al.* 1984)** in 1984. In 1988, a new protozoan species, N. caninum, was proposed under a new genus named Neospora **(Dubey *et al.* 1988)**. The parasite is recognized as an important cause of reproductive problems. Worldwide, with widespread occurrence of neosporosis in beef cattle, dairy cattle, or both **(Waldner *et al.* 2001)**. The protozoan parasite *Neospora caninum* has become increasingly recognized as an important cause of abortion in dairy and beef cattle **(Gulay *et al.* 2006)**. Domestic canids are the natural definitive host for *Neospora caninum* **(Dubey, 2003)**. The infected dog sheds unpopulated oocysts in the

environment for 5 to 17 days after the ingestion of tissue cysts **(McAllister *et al.* 1998)**. Intermediate hosts (cattle) ingest oocysts that are found in contaminated food and water. Sporozoites are released in the intestinal tract where they penetrate cells and become tachyzoites (a rapidly dividing asexual phase). Tachyzoites divide and quickly spread to other host cells, which they invade and often destroy. Tachyzoites have been found in neural cells, macrophages, fibroblasts, vascular endothelial cells, hepatocytes, and muscular cells including those of myocardium and the placenta in pregnant cows **(Dubey and Lindsay, 1996).** The tachyzoites can be transmitted vertically from a dam through the placenta to the fetus **(Shivaprasad *et al.* 1989 and Moore *et al.* 2014).** In neural cells, tachyzoites can transform into bradyzoite when a strong immune response is mounted against the protozoa elsewhere in the body. The bradyzoite form tissue cysts for protection; they remain latent until the immune system of the intermediate host is suppressed **(Dijkstra *et al.* 2001)**. Cysts have been found in the brain, spinal cord, and retina. Tachyzoites in placental tissue (cysts), when consumed by a dog, implant in the gastrointestinal tract where they mature, begin to shed oocysts, and complete the horizontal transmission cycle **(Uggla *et al.* 1998)**. Infected dogs excrete oocysts in their faeces, which may then be ingested by intermediate hosts such as cattle, sheep and goats **(Bjerkas *et al*.1984)**. The characteristics of the oocysts of *N. caninum* are quite similar to those of oocysts of *Hammondia heydorni* from dog feces and of Toxoplasma gondii from cat feces **(Lindsay *et al* 1999)**. Furthermore, the agents' tachyzoites and bradyzoite appear similar under a light microscope, but they can be distinguished under an electron microscope by the number, appearance, and location of their rhoptries, that they are different protozoa **(Almeria *et al.* 2002**). The principal route of infection in cattle is transplacental (vertical) transmission **(Barr *et al.* 1994)** and the same cow can pass the infection to multiple offspring. The dog is the "definitive host 'of Neospora, and cattle are "intermediate hosts". The probability of a seropositive dam producing a calf that is seropositive prior to consumption of colostrum has been widely reported as ranging between 81% and 100%. Abortion due to neosporosis may occur over several generations **(Innes *et al.*, 2002)**.



After Dunkel, (2001)

There is no known effective and economic treatment for bovine neosporosis **(Dubey, 2003)**. However, vaccination of cattle with inactivated *N. caninum* tachyzoites was reported to prevent cattle from abortions **(Kim *et al*., 2002)**. The aim of the present study is to evaluate the significance of neosporosis in animals in different localities in Egypt.

Materials and methods:

Blood samples were collected from 350 female animals (cows, ewes and buffalo dams) in 6 governorates in Egypt (Table 1) over a full one-year period **(January to December 2016).** All of the herds had previous history of abortion with no determined reasons for that. Jugular vein blood was collected in vacutainer tubes and was transferred to the Protozoal Lab. of Animal Reproductive Research Institute. After centrifugation at 3000 rpm × 15 min, sera were separated and stored at -20 0C until analysis.

Table (1): Number of blood samples among different animals in different governorates of Egypt.

Localities	No. of blood samples	Samples of different animals		
		Cows	ewes	Buffalo-cows
Fayoum	30	10	17	3
Giza	75	24	39	12
Beni-sweif	70	25	34	11
Menia	40	7	27	6
Aswan	80	27	28	25
Sohag	55	11	24	20
Total	350	104	169	77

Serum samples were screened for specific antibodies to *Neospora caninum* using commercially available diagnostic indirect ELISA kit in serum, plasma or milk for multispecies (ID. Vet Louis Pasteur. Grabeis, France) using X check software program. All control tests were performed in duplicate. The diluent, wash solution and dilution buffer were provided by ID.vet. The optical density (OD) values of the wells were read with ELISA reader (Titertek Multiskan plus MK II), at a wavelength of 450 nm. The presence and absence of antibody to *Neospora caninum* were determined by sample to positive (S/P) ratio for each sample.

RESULTS

Antibodies to *Neospora caninum* were detected in 79 out of 350 blood samples among different animals in 6 governorates in Egypt with a percentage of 22.57 % (Table 2). Seropositive cows were found in 34 out of 104 (43.04%); 32 out of 169 ewes (40.51%) and 13 out of 77 buffalo dams with a percentage of 16.46%.

Table (2): Number of seropositive serum samples among different animals in different governorates of Egypt.

Localities	No. of blood samples	No. of positive samples %	Positive samples of different animals		
			Cows	ewes	Buffalo-cows
Fayoum	**30**	**3 (10%)**	**2 (6.66%)**	**1 (3.33%)**	**--**
Giza	**75**	**12 (16%)**	**7 (9.33%)**	**3 (4%)**	**2 (2.67%)**
Beni-sweif	**70**	**25)35.71%)**	**12 (17.14%)**	**9 (12.86%)**	**4 (5.71%)**
Menia	**40**	**8 (20%)**	**3 (7.5%)**	**3 (7.5%)**	**2 (5%)**
Aswan	**80**	**20 (25%)**	**7 (8.75%)**	**10 (12.5%)**	**3 (3.75%)**
Sohag	**55**	**11 (20%)**	**3 (5.45%)**	**6 (10.91%)**	**2 (3.64%)**
Total	**350**	**79 (22.57%)**	**34 (43.04%)**	**32 (40.51%)**	**13 (16.46%)**

From the 350 blood sampled, 124 had a previous record of abortion. Of these, 30 (24.19%) were seropositive cows 15 (50%); ewes were found in 11 (36.67%) and buffalo-cows were found in 4 (13.33%) (Table 3).

Table (3): Seroprevalence of *N. caninum* in aborted samples belonging to animals in different governorates in Egypt.

Localities	No. of blood samples	No. of positive samples %	Positive samples of different animals		
			cows	ewes	Buffalo-cows
Fayoum	12	3/12 (25%)	2 (16.67%)	1 (8.33%)	--
Giza	15	7/15 (46.67%)	3 (20%)	3 (20%)	1 (6.67%)
Beni-sweif	27	5/27 (18.52%)	2 (7.41%)	2 (7.41%)	1 (3.70%)
Menia	19	4/19 (21.05%)	2 (10.53%)	1 (5.26%)	1 (5.26%)
Aswan	35	6/35 (17.14%)	3 (8.57%)	2 (5.71%)	1 (2.86%)
Sohag	16	5/16 (31.25%)	3 (18.75%)	2 (12.5%)	--
Total	124	30/124 (24.19%)	15 (50%)	11 (36.67%)	4 (13.33%)

The prevalence of *N. caninum* was marked higher in the aborted animals than in the non-aborted animals. There was marked difference between aborted and non-aborted groups. (Table 4).

Table (4): The Seroprevalence of *N. caninum* in normal and aborted animals in different governorates in Egypt.

Localities	Non-aborted animals (%)	Aborted animals* (%)
Fayoum	18/0 --	12/3 (25%)
Giza	60/5 (8.33%)	15/7 (46.67%)
Beni-sweif	43/20 (46.51%)	27/5 (18.52%)
Menia	21/4 (19.05%)	19/4 (21.05%)
Aswan	45/14 (31.11%)	35/6 (17.14%)
Sohag	39/6 (15.38%)	16/5 (31.25%)
Total	226/49 (21.68%)	124/30 (24.19%)

DISCUSSION

The effects of neosporosis on productivity of farm animals include reproductive losses, reduction in milk production, premature culling and reduced weight gain is more common in intensively managed dairy farms **(João, *et al.* 2005**). Abortion due to Neospora is typically occurs in mid gestation with a mean pregnancy of 5.5 months (range 3.5-8 months) and may occur throughout the year. This is different from most causes of bovine abortion which occur during late gestation. Abortion storms may occur with multiple abortions over a period of 1-2 months **(Brad, 2001and Klauck, *et al.* 2016).** All females that have aborted should be screened by serological assay, screened for *N. caninum* antibodies. Current ELISA technology expedites and automates the process of herd screening, without compromising quality. Neospora Ab test, offers rapid detection of antibodies against *N. caninum* in samples of bovine and ovine serum **(Lyon, 2010)**. The Seroprevalence of N. caninum infection (22.57%) in farm animals investigated was included the higher incidence (43.04%) positive cases in dairy cows, followed by (40.51%) positive cases in ewes and (16.46%) positive cases in buffalo-cows. These data confirm the high prevalence of the infection in bovine, and the insidious way of *N. caninum* diffusion may maintain endemic the disease in farm animals, with consequent periodic abortions if no preventive measures are adopted. These results agree with **Otranto *et al.* (2003)** who found an increase of the *N. caninum* infection in Italy. The higher incidence was (35.71%) in Beni-sweif Governorates followed by (25%) in Aswan; (20%) in Menia and Sohag; (16%) in Giza and lowest (10%) in Fayoum. The females that were examined in this study belonged to small-scale farms and any low incidence of the disease could affect the farmer's economic situation, although abortion cases are sporadic. The Seroprevalence of *N. caninum* in aborted samples among different animals in Egypt was (24.19%) higher in aborted cows (50%) followed by aborted ewes (36.67%) and then buffalo-cows (13.33%). These findings agree with pens may be sufficient to reduce the risk of neosporosis transmission from dogs **(Moore, *et al.* 2014)**. further studies to clarify the impact of neosporosis on the animal industry and losses due to clinical neosporosis in livestock in Egypt is recommended parallel with application of the control strategies for dairy farms. In the present study, the prevalence of *N. caninum* was higher in the aborted females than in the non-aborted. The incidence was (24.19%) in aborted females while it was (21.68%) in non-aborted females. These results agreed with **Kim, *et al* (2002)**. Abortion is thought to be associated with immunosuppression and increased risk of subsequent foetal loss

(Moen, *et al.* 1998 and Sager, *et al*, 2001). Herds that already have neosporosis may show sporadic neosporosis abortions for several years, even if transmission from dogs is completely eliminated. This is because infections in farm animals may last for life, and infections may be transmitted from chronically-infected females to their fetuses **(McAllister, *et al* 1998)**. Most of these newly born will appear perfectly normal. Infected animal can be identified by a blood test that detects antibodies to Neospora **(Stefano, *et al*. 2016).** Culling infected females can be recommended. In the meantime, transmission from dogs can be prevented, and then the incidence of abortion in the herd should gradually drop to "normal" over the course of several years, without any testing or special culling. Prevention of fecal contamination of feed is essential. Under most circumstances, bitches on dairies should be spayed. Stray dogs should be controlled **(Almeria, *et al*. 2002).**

Control strategies:

• Blood testing of breeding stock. The ideal time is in the first two weeks of life.

•Positive animals not used to breed replacement stock.

• Dispose of all placentae and aborted fetuses by bagging and burning.

• Do not allow dogs to eat any access of placentae, fetuses or neonatal carcasses.

• Maintain good biosecurity of feed and water sources, in particular to prevent contamination by dogs.

• If there are areas of pasture where dogs have access, do not use to graze cows and preferably do not use for silage.

• Treat all dogs on farm with Clindamycin (a wormer) to protect against infection.

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