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SEROLOGICAL DIAGNOSIS OF *NEOSPORA CANINUM* INFECTION IN SOME DOMESTIC ANIMALS FROM EGYPT

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SUMMARY

This study investigates the presence of antibodies to *N. caninum* in cattle, sheep, goats and dogs from Egypt. Sera from the tested animals were examined using a direct agglutination test incorporating mercaptoethanol and formalin-preserved whole *N. caninum* tachyzoites as antigen. Anti-*N. caninum* antibodies were found in 18 out of 111 aborted cattle (16.2 %). Abortion occurs during the third to ninth month of gestation period. Three of the 26 non-aborted cattle (11.5 %) were seropositive. Statistical analysis indicated that anti- *N. caninum* antibodies occurred more in the aborted than in the non-aborted group (slightly significant). Seventy three of 202 sheep (36.1 %) and 31 of 88 (35.2%) goats recorded antibodies for *N. caninum*. Eight out of 29 (27.6 %) stray dogs had antibodies agaist *N. caninum*. The present communication is the first nation wide survey on serological evidence of *N. caninum* antibodies in different life stock and dogs in Egypt.

INTRODUCTION

Neospora caninum is a protozoan parasite that was first recognized in dogs with neurological signs (Dubey et al., 1988). Subsequently the parasite was incriminated as a major cause of abortion in cattle all over the world (Dubey and Lindsay, 1996).

Natural infection with *Neospora species* was reported from sheep, goats and horses (Dubey and Lindsay, 1996). Recently the domestic dog was found to be the definitive host of *N. caninum* since it excretes environmentally resistant oocysts in faeces after ingestion of tissue cysts (Mc Allister et al., 1998). Since accurate diagnosis of *N. caninum* is key for understanding of the

epidemiology of neosporiosis, several reports were published from different countries on the seroprevalence of the parasite in farm animals (Hemphill et al., 2000).

In Egypt there are only two published reports on the seroprevalence of anti-Neospora caninum antibodies in camels (Hilali et al., 1998) and water buffaloes (Dubey et al., 1998). Similar studies on other farm animals are non-existent. Therefore, this research was undertaken to estimate the seroprevalence of *N. caninum* antibodies in cattle, sheep, goats and dogs.

MATERIALS AND METHODS

1- Animals and Blood samples

Blood samples were obtained from 111 aborted and 26 non-aborted cattle. Both groups were chosen from 3 dairy Holestein cattle farms (Table 1). All investigated cattle varied in age from 3-7 years old. The gestational age of abortion varied in these farms from 3-9 months. The non-aborted cattle were pregnant at a gestational age varying from 3-9 months. Serum samples were collected immediately after abortion from aborted cattle. While, the samples were collected from the non- aborted cattle during their gestational period.

Two hundred and two sheep were sampled from farms in Delta (183 samples) and in the suburbs of Cairo (19 samples). The age of sheep varied from 1-3 years. A total of 88 goat blood samples were obtained from farms in the suburbs of Cairo (14), Delta (24) and Sinai Peninsula (50). The goats varied in their ages from 1-3 years.

All the collected dog samples (29) were obtained from stray dogs living in dairy farms in the suburbs of Cairo city. Five ml of blood was obtained from each animal and serum was separated and kept at -20°C until used.

2- Serological analysis

Sera were tested by using *Neospora caninum* agglutination test (NAT) kit (Vetoquinol, France) as described by Romand et al. (1998). The used antigen was formalin-preserved whole tachyzoites of *N. caninum* incorporated with mercaptoethanol. The tested sera in addition to the positive and negative control sera were used at a dilution of 1:40. The samples which showed a diffused precipitation in the bottom of the wells were considered positive while negative samples showed a spot precipitate. The positive samples were serially diluted beginning from 1:40 to 1:640 for the determination of end point titer.

RESULTS

Abortion in cattle with anti-*Neospora* antibodie occurred at a gestational age varying from 3-months. As illustrated in table 1, serological examination of 111 aborted cattle showed that onl

3 had anti-*N. caninum* antibodies (16.2%). The stribution of these serologically positive cattle as 7 (21.9%), 8 (20%), 3 (7.7%) three different ırms. On the other hand, serological examina-on of pregnant non-aborted cattle chosen from ıe same farms indicated that 3 cattle (11.5%) 'om a total number of 26 were positive for *N. aninum*. One of these positive cattle was from ırm I and two were from farm II. None of the se-ɔcted 9 cattle in farm III had antibodies against *I. caninum*. The end point titers among 18 sero-ogical positive aborted cattle were 1:40 (11), :80 (2), 1:160 (3) and 1;640 (2) (Table 4). The ɔerologically positive non-aborted cattle had end ɔoint titer of 1:40 (two cattle) and 1:80 (one cow). As shown in table 2 statistical analysis indicated that anti-*N. caninum* antibodies occurred more in the aborted than in the non-aborted group (slightly significance).

Table 3 showed that 73 out of 202 examined sheep (36.1%) had antibodies against *N. caninum*. Sixty seven sheep out of 183 (36.6%) in Delta and 6 of 19 sheep (31.6 %) from Cairo recorded positive results for *N. caninum* antibodies. Five out of the 14 goat samples (35.7%) from Cairo, 8 out of 24 goats (33.3%) in Delta and 18 out of 50 goats (36%) from Sinai reported positive results for *Neospora caninum*. The total percentage of infected goats was 35.2%. Eight

Table 1: Distribution of serologically positive cattle in the 3 farms .

Farm No.	Serological results of aborted cattle					Serological results of pregnant non aborted cattle				
	Total Number	No. of Positive	%	G.A. (month	No.	No. of examined	No. of Positive	%	G.A. (month	No.
I	32	7	21.9	5	3	7	1	14.3	4	1
				6	2					
				7	1					
				8	1					
II	40	8	20	4	1	10	2	20	6	1
				5	2				8	1
				6	2					
				7	2					
				8	1					
III	39	3	7.7	5	1	9	Zero	0		
				6	1					
				7	1					
Total	III	18	16.2			26	3	11.5%		

Table 2: chisquare at degree of freedom (3x6) at P> 0.05 of the examined cattle.

GA (month)	Aborted cattle			Non aborted			Total examined
	Positive	Negative	Total	Positive	Negative	Total	
3	0	4	4	0	3	3	7
4	1	12	13	1	4	5	18
5	6	25	31	1	4	5	36
6	5	27	32	0	5	5	37
7	4	16	20	0	2	2	22
8	2	9	11	1	3	4	15
9	0	0	0	0	2	2	2
Total	18	93	111	3	23	26	137

Chisquare all 21.636

Table 3: Results of serological examination of sheep, goats and dogs for *N. caninum*.

Animal	Location	Total Number	Positive	Percentage
Sheep	Delta	183	67	36.6
	Cairo	19	6	31.6
Total sheep		202	73	36.1
Goat	Cairo	14	5	35.7
	Delta	24	8	33.3
	Sinai	50	18	36
Total goat		88	31	35.2
Dog	Cairo	29	8	27.6

Table 4: End point titers for *N. caninum* antibodies in seropositive cattle, sheep, goats and dogs.

	Total	1:40		1:80		1:160		1:320		1:640	
		No	%	No	%	No	%	No	%	No	%
Aborted cattle	18	11	61.11	2	11.11	3	10.66	0	0	2	11.11
Non aborted cattle	3	2	66.66	1	3.8	0	0	0	0	0	0
Sheep	73	61	83.56	10	4.9	0	0	2	2.73	0	0
Goat	31	28	90.32	1	1.3	2	6.45	0	0	0	0
Dog	8	4	50	4	13.8	0	0	0	0	0	0

ay out of 29 dogs (27.6 %) has antibodies ainst *N. caninum*. The distribution of the end int titers for *N. caninum* antibodies in the serositive samples is illustrated in Table 4. Among seropositive sheep, 61, 10 and 2 showed end int titer 1:40, 1:80 and 1:320 respectively. hile, 28, 1, 2 out of 31 *N. caninum* seropositive ats showed titer 1:40, 1:80 and 1: 160 respecely. The seropositive dogs showed end point er of 1:40 (4) and 1:80 (4).

ISCUSSION

here are several reports on the seroprevalence of ntibodies against *N. caninum* in farm animals rom different countries by different serological sts (Hemphill et al. 2000). In 1998, two indeendent papers described *N. caninum* direct aglutination test according to the principal of the *oxoplasma gondii* modified agglutination test Romand et al., 1998 and Packham et al., 1998). The test described by Romand et al. (1998) emloyed tachyzoites of the canine NC-1 isolate vhile Packman et al. (1998) used a bovine isoate. This study used the bovine isolate (Packham t al., 1998). NAT was proved to be highly specific and sensitive in diagnosis of *N. caninum* in animals infected with related parasites including *T. gondii* (Romand et al., 1998). Because of its simplicity and visibility, the NAT has the potential of being used for analysis of sera from a variety of species, possibly replacing the IFAT (Huong et al., 1998). Hence, in this study we used NAT as a serological test for detection of *N. caninum* antibodies.

The present study showed that 18 out of 111 (16.2 %) aborted cattle and 3 of the 26 (11.5 %) non aborted cattle had antibodies for *N. caninum*. Statistical analysis indicated that anti-*N. caninum* antibodies occurred more in the aborted than in the non-aborted group (slightly significant). This indicates thet *N. caninum* is one of the abortificient agents in cattle. This agree with that reported by Anderson et al., (1991) in USA and by Hemphill et al. (2000) in different European countries, Newzealand and Australia.

The incidence of *N. caninum* in cattle was also studied in different countries, Vietnam (5.5%, Huong, et al., 1998); France (5.6%, Ould-Amrouche et al., 1999); Korea (23%, Bae et al., 2000); Sweden (7%, Bjorkman et al., 2000); Brazil (14.09%, Gondim et al., 1999); Thailand (6%, Suteeraparp et al., 1999) and Spain (30.6%, Mainar et al., 1999).

The present study recorded that in Delta and Cairo, 73 of 202 sheep (36.1% were seropositive for *N. caninum*. Thirty one of 88 (35.2%) goats recorded antibodies for *N. caninum*. Little is known about the seroprevalence of *N. caninum* in sheep and goats. However, Barr et al. (1992) found it in tissue of goats.

Stray dogs showed *N. caninum* antibodies in

27.6 %. Cheadle et al. (1999) in Alabama recorded that 11.5% of examined dogs had *N. caninum* antibodies. Wouda et al. (1999) found that 23.6% of dogs in Netherland had been exposed to the parasite. *N. caninum* antibodies were reported from dogs in Australia (9%), Urguay (20%), Falkland Islands (0.2%), and Belgium (11%) (Barber et al., 1997 a and b). Reichel et al., (1998) found seroprevalence of 22% in dogs from NewZealand, while it was 29% from Italy (Cringoli et al., 1996). The above mentioned variation in seroprevalence rates might be due to the type of the examined dogs (stray or indoor dogs, dogs in dairy farms or in urban areas). The variation between the end point titers in each animal species might be attributed to the difference in age, time of infection and the immune system of the examined animals.

The obtained results from this study could be a preliminary guide for the presence of *N. caninum* in Egyptian animals. Isolation and identification of *N. caninum* from these animals is considered an important step for confirming the role of *N.* caninum in inducing abortion and still birth in Egyptian animals.

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