Am. J. Trop. Med. Hyg., 80(2), 2009, pp. 263–267

Short Report: Prevalence of *Neospora caninum* and *Toxoplasma gondii* Antibodies in Northern Egypt

Hany M. Ibrahim, Penglong Huang, Tarek A. Salem, Roba M. Talaat, Mahmoud I. Nasr, Xuenan Xuan, and Yoshifumi Nishikawa*

National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido, Japan; Genetic Engineering and Biotechnology Institute, Minufiya University, Sadat City, Egypt

Abstract. In view of the worldwide importance of *Toxoplasma gondii* and *Neospora caninum* and the limited data on the seroprevalence of these parasites in Egypt, this study aimed to estimate the prevalence of anti-*T. gondii* and anti-*N. caninum* antibodies in rabbits, cattle, and humans. We used ELISA methods based on surface antigen 2 of *T. gondii* (*Tg*SAG2t) and surface antigen 1 of *N. caninum* (*Nc*SAG1t). High seroprevalence of *T. gondii* (51.49%) was detected in pregnant women, and antibodies to *N. caninum* were also detected in human samples (7.92%). Anti-*T. gondii* or *N. caninum* antibodies were detected in cattle (*Tg*SAG2t: 10.75%; *Nc*SAG1t: 20.43%). In rabbits, only one sample was *N. caninum* positive (1.85%). The high prevalence of toxoplasmosis and neosporosis in cattle affects the development of the livestock industry and is also an important infective source for human infection in Egypt.

Toxoplasma gondii and *Neospora caninum* are two closely related protozoan parasites distributed worldwide. Both organisms have an indirect life cycle with carnivores as the definitive hosts and can infect a wide range of animal species. In the host, *T. gondii* can cause abortion or neonatal mortalities.[1] The organism is estimated to infect 4–77% of the human population.[2] Although it is not normally a significant problem for healthy individuals, *T. gondii* infection can be life threatening for infants infected congenitally and immunocompromised and immunodeficient patients (e.g., AIDS patients, cancer patients, and organ transplant recipients), as a result of either acute infectionor reactivation of infection.[3–6] Toxoplasmic encephalitis is a life-threatening central nervous system infection observed in the later stages of HIV infection.[7] In animals, *T. gondii* infection not only results in significant reproductive losses, and hence economic losses, but also has implications for public health because consumption of infected meat or milk can facilitate zoonotic transmission.[8] Neosporosis, caused by the protozoan *Neospora caninum*, is an important cause of bovine abortion[9] and neurologic alterations in dogs.[10] It can also cause abortion or neonatal mortality in other animal species, such as sheep, goats, horses, and deer.[11] Moreover, antibodies against *N. caninum* were detected in humans.[12,13] However, there are no reports about the clinical implications of *N. caninum* in humans because the parasites have not been detected or isolated from human tissues.

Serologic testing is an important method for detecting these parasitic infections, and includes immunofluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA), competitive-inhibition ELISA, Western blotting, and direct agglutination test (DAT) using intact tachyzoite or tachyzoite-derived antigens.[14,15] However, the use of whole tachyzoites or tachyzoite-derived antigens may result in false positives because of cross-reaction with other closely related parasites.[16] Therefore, it is necessary to develop a reliable, sensitive, and specific diagnostic test using parasite-specific antigens. The molecular search for diagnostic antigens for *T. gondii* and *N. caninum* infection has been focused on the identification of immunodominant antigens that are recognized by sera from animals infected with geographically distant isolates and from both acute and chronically infected animals. Surface antigen 2 of *T. gondii* (*Tg*SAG2), expressed either in *Escherichia coli* or insect cells, was validated as a useful antigen and promises a highly sensitive and specific ELISA.[17,18] Surface antigen 1 of *N. caninum* (*Nc*SAG1) is an important candidate for developing a diagnostic reagent for neosporosis.[19,20]

In previous surveys from Egypt, *T. gondii* antibodies were found in 27.3% of sera from 600 asymptomatic pregnant women,[21] 47% of 108 chickens,[22] 15.7% of 19 ducks,[23] and 65.6% of 121 donkeys.[24] Anti-*T. gondii* and *N. caninum* antibodies were detected in 17.4% and 3.6% of 166 camels, respectively.[25] A total of 51 of 75 (68%) water buffalo sera had antibodies to *N. caninum*.[26]

Economically, toxoplasmosis and neosporosis are considered important diseases in animals, and toxoplasmosis causes a variety of clinical manifestations in humans. Hence, our objective was to estimate the prevalence of anti-*T. gondii* and anti-*N. caninum* antibodies in rabbits, cattle, and humans by ELISA. We studied the serum of 101 human samples from Dakahlia province, Mansoura City (northeast of Delta), and 93 cattle samples and 54 rabbit samples from Sharkia Province (east of Delta).

The *N. caninum* (NC-1 strain) and *T. gondii* (RH strain) tachyzoites were maintained on monkey kidney adherent fibroblasts (Vero cells) cultured in Eagle minimum essential medium (EMEM; Sigma, St. Louis, MO) supplemented with 8% heat-inactivated fetal bovine serum. For the purification of tachyzoites, parasites and host-cell debris were washed in cold phosphate-buffered saline (PBS), and the final pellet was resuspended in cold PBS and passed through a 27-gauge needle and a 5.0-μm-pore filter (Millipore, Bedford, MA).

Blood samples were collected from the brachial vein of 101 pregnant women, (20–35 years of age, 6–18 weeks of gestation) at private clinical laboratories in Dakahlia Province, Mansoura City (northeast of Delta). Blood samples were collected from the jugular or caudal vein by local veterinary practitioners from 93 cattle at a cattle veterinary station and 54 albino rabbits samples at San El-hagr rabbit farm, Sharkia Province (east of Delta). See Figure 1 for a map of the sampling area. Blood

* Address correspondence to Yoshifumi Nishikawa, National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan. E-mail: nisikawa@obihiro.ac.jp

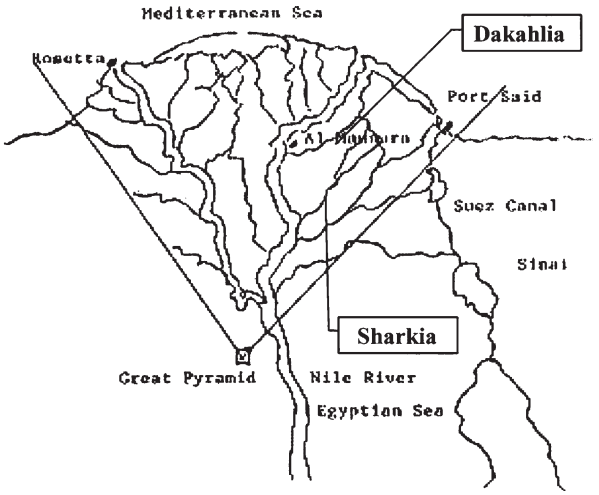

FIGURE 1. Map of sampling area. Serum samples were collected from 101 pregnant women at private clinical laboratories in Dakahlia Province, Mansoura City (northeast of Delta). Animal samples were collected from 93 cattle at a cattle veterinary station and 54 albino rabbits at San El-hagr rabbit farm, Sharkia Province (east of Delta).

samples were centrifuged at 1,000 *g* for 10 minutes, and the serum was collected and stored at –20°C.

The template DNA for polymerase chain reaction (PCR) was extracted from tachyzoites of the *T. gondii* RH strain and *N. caninum* Nc-1 strain.[11,14,16,27] Two oligonucleotide primers, 5′-ACGAATTCGTCCACCACCGAGACG-3′ and 5′-ACGAATTCTTACTTGCCCGTGAGA-3′, which correspond to amino acids 75 to 221, were used to amplify the truncated *SAG2* (*TgSAG2t*) gene, without sequences encoding a highly hydrophobic signal peptide and C terminus by PCR.[14,28] The truncated *NcSAG1* (*NcSAG1t*) gene, without sequence encoding a hydrophobic signal peptide and a C ter-minus, was amplified by PCR with two primers 5′-ACGAATTCATCAGAAAAATCACCT-3′ and 5′-ACGAATTC-GACCAACATTTTC AGC-3′, which correspond to amino acids 65 to 333.[16] The *TgSAG2t* gene or *NcSAG1t* gene was inserted into *Eco*RI site of the bacterial expression vector, pGEX-4T-3 (Promega, Madison, WI). Each resulting plasmid was designated as either pGEX/TgSAG2t or pGEX/NcSAG1t. pGEX/TgSAG2t or pGEX/NcSAG1t was expressed as glutathione *S*-transferase (GST) fusion protein (GST-TgSAG2t or GST-NcSAG1t) in *E. coli* (DH5α strain), and the proteins were purified by glutathione Sepharose 4B according to the method of Chahan and others.[16]

ELISA was performed according to modified procedures described previously.[14,16,17] The plates were coated using the recombinant antigens (GST-*Tg*SAG2t, GST-*Nc*SAG1t, or GST, 5 μg/mL), produced as described earlier, in a coating buffer (50 mmol/L carbonate) and incubated overnight at 4°C. After washing once with washing buffer (PBS containing 0.05% Tween 20), the plates were blocked with blocking solution (PBS containing 3% skim milk) at 37°C for 2 hours. After washing once with washing buffer, 50 μL of serum diluted (1:100) in blocking solution was added to duplicate wells for each sample and incubated at 37°C for 1 hour. After washing six times with washing buffer, the plates were incubated with 50 μL of horseradish peroxidase (HRPO)-conjugated goat anti-bovine immunoglobulin G plus IgA and IgM (Bethyl Laboratories, Montgomery, TX), HRPO-conjugated goat anti-rabbit immunoglobulin G (Bethyl Laboratories), and HRPO-conjugated goat anti-human immunoglobulin G (Sigma) diluted in blocking solution (1:4,000) per well at 37°C for 1 hour. After washing six times with washing buffer, the plates were incubated with 100 μL substrate 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) in an ABTS buffer (0.1 mol/L citric acid, 0.2 mol/L sodium phosphate) per well at room temperature for 1 hour. The absorbance at 405 nm was measured using a microplate reader (TECAN Sunrise, Grödig, Austria). The ELISA result was determined by the difference in mean optical densities at a value of 405 nm (OD_{405}) between the recombinant antigen (*Tg*SAG2t or *Nc*SAG1t) and the GST protein. The cut-off values were determined as the OD_{405} value for *T. gondii*– or *N. caninum*–negative sera plus 3 SD—*Tg*SAG2t: 0.039 and *Nc*SAG1t: 0.211 in humans (N = 13), *Tg*SAG2t: 0.02 and *Nc*SAG1t: 0.042 in cattle (N = 15), and *Tg*SAG2t: 0.041 and *Nc*SAG1t: 0.031 in rabbits (N = 10). The negative sera from our sera stock were tested and confirmed negative by ELISA, DAT, and IFAT.

Slides were spotted with whole *N. caninum* (NC-1 strain) tachyzoites. The purified tachyzoites were washed three times in PBS (25 mmol/L $NaPO_4$–150 mmol/L NaCl; pH 7.2) and diluted to 10^6/mL. One drop of the solution was placed in each of the 12 wells per slide and allowed to dry at 37°C. The cells were fixed with 80% acetone–20% methanol.

Sera used in the IFAT were diluted 1:100 in PBS, and 20 μL of each sample was added to a well containing tachyzoites and incubated in a humidified chamber at 37°C for 30 minutes. The sera were removed, and each well was rinsed and washed for 10 minutes with rinse buffer (25 mmol/L Na_2CO_3, 100 mmol/L $NaHCO_3$, 36 mmol/L NaCl; pH 7.4). Alexa Fluor 488 goat anti-human immunoglobulin G (IgG) and Alexa Fluor 488 goat anti-mouse immunoglobulin G (IgG) (Invitrogen; Molecular Probes, Eugene, OR) diluted 1:100 in PBS were placed in each well. The slides were incubated and washed as described above, overlaid with mounting medium (50% glycerol–50% rinse buffer) and a coverslip, and viewed at ×63 magnification by confocal fluorescence microscopy. For controls, one well on each slide was tested with *N. caninum*–negative control serum. Moreover, another well was tested with *N. caninum*–positive mice control serum to confirm reactivity.

The prevalence of *T. gondii* and *N. caninum* in humans (pregnant women), cattle, and rabbits from the northeastern and eastern Delta regions is summarized in Tables 1 and 2, respectively. The results of the ELISA for detecting the antibodies against the recombinant *Tg*SAG2t showed high seroprevalence (51.49%) in pregnant women, 10.75% in cattle, and 0% in rabbit samples. Antibodies against the recombinant *Nc*SAG1t were detected in pregnant women (7.92%), cattle (20.43%), and rabbits (1.85%). Mixed infection of both

TABLE 1
Seroprevalence of *T. gondii* infection in humans, cattle, and rabbits

Regions	Samples	Numbers of sample	Numbers of positive samples	Seroprevalence (%)
Dakahlia	Human	101	52	51.49
Sharkia	Cattle	93	10	10.75
Sharkia	Rabbit	54	0	0

TABLE 2
Seroprevalence of *N. caninum* infection in humans, cattle, and rabbits

Regions	Samples	Numbers of sample	Numbers of positive samples	Seroprevalence (%)
Dakahlia	Human	101	8	7.92
Sharkia	Cattle	93	19	20.43
Sharkia	Rabbit	54	1	1.85

T. gondii and *N. caninum* was detected in human sera (5.94%, $N = 6$). On the other hand, the mixed infection was not observed in either cattle or rabbit sera. Because human neosporosis is controversial, we minutely confirmed the seroreactivity of *N. caninum*–positive human samples examined by ELISA. The IFAT showed that all human samples ($N = 8$) reacted with the *N. caninum* tachyzoite. The positive human sera showed obvious tachyzoite staining like the staining with positive mouse serum (Figure 2).

The proportion of women at risk of acquiring the *Toxoplasma* infection during pregnancy in many countries, including Egypt, is not well known. Primary infection with *Toxoplasma* during pregnancy may lead to severe complications and may result in the death of the fetus.[29,30] Our study showed that there was a high seroprevalence of *T. gondii* (Table 1; seroprevalence was 51.49%) in pregnant women in Dakahlia Province, Mansoura City, Egypt. High percentages of *T. gondii* infection were reported in Jordan[29,31] (54% and 58.2%) and in Mexico[32] (44.9%). Also, Zuber and Jacquier[33] reported that serologic evidence indicated that human infections are common in many parts of the world. Human neosporosis is a controversial question now because *N. caninum* was not detected or isolated from human tissues. In our study, *N. caninum*–specific antibodies were detected in pregnant women (Table 2; Figure 2; seroprevalence was 7.92%). These results are consistent with previous studies conducted in the United States[12] and Brazil.[13] This seroprevalence suggests human exposure to *N. caninum*, but further study is needed to determine the extent and significance of exposure. Because dogs are definitive hosts and excrete oocysts in their feces, the potential for human exposure to *N. caninum* is high.[34] The infection of healthy individuals by *N. caninum* may follow a course similar to that of *T. gondii*, where the vast majority of infections are asymptomatic.[35] Testing tissues and fluids from immunocompromised individuals and fetuses with suspected toxoplasmosis for *N. caninum* may show that subpopulations of these patients are infected with *N. caninum*. Mixed infection of both *T. gondii* and *N. caninum* was detected in 5.94% ($N = 6$) of the human sera tested, indicating concomitant infection of *T. gondii* and *N. caninum* in those women.

The prevalence of *T. gondii* and *N. caninum* in cattle and rabbits in Sharkia Province, Egypt, was detected by ELISA with *Tg*SAG2t and *Nc*SAG1t as coated recombinant antigens. Anti-*T. gondii* or -*N. caninum* antibodies were detected in cattle (*Tg*SAG2t: 10.75% in Table 1; *Nc*SAG1t: 20.43% in Table 2), whereas only one sample was *N. caninum* positive in rabbits (*Tg*SAG2t: 0% in Table 1; *Nc*SAG1t: 1.85% in Table 2). No mixed infection of both *T. gondii* and *N. caninum* was detected in the animal sera tested. These results suggest that the identification of recombinant *Tg*SAG2t and recombinant *Nc*SAG1t could distinguish between toxoplasmosis and neosporosis. The high prevalence of toxoplasmosis and neosporosis in cattle not only affects the development of the livestock industry but is also an important infective source for human toxoplasmosis.

There are three possible routes by which the host could become infected with *T. gondii* or *N. caninum*: ingestion of sporulated oocysts, ingestion of bradyzoite cysts in the tissues of intermediate hosts, or vertical transmission.[1] Many risk factors need to be studied to understand the high percentage of parasitic infection. In Egypt, consumption of grilled lamb (undercooked) is very high. Sheep are reared outdoors, which puts them at greater risk of environmental exposure than animals reared indoors.[36] It is these trends that may increase the exposure to parasites because lamb has a greater potential as an infection source than beef or poultry. Another risk factor associated with seropositivity is contact with soil-harboring oocysts from wild homeless cats and dogs, which also may be responsible for the high infection rates. If the infections of these parasites increase and spread among domestic animals, contamination of the water and soil will also increase. In Egypt, *T. gondii* has been reported in chickens and ducks,[22,23] in horses,[37] and in donkeys,[24] and both *T. gondii* and *N. caninum* have been reported in water buffalo and camels.[25,26]

In conclusion, our study indicated that these diseases may be widely distributed and present the threat of an epidemic in Delta, Egypt, with high seropositivity in humans and cattle. Recombinant *Tg*SAG2t and *Nc*SAG1t are good

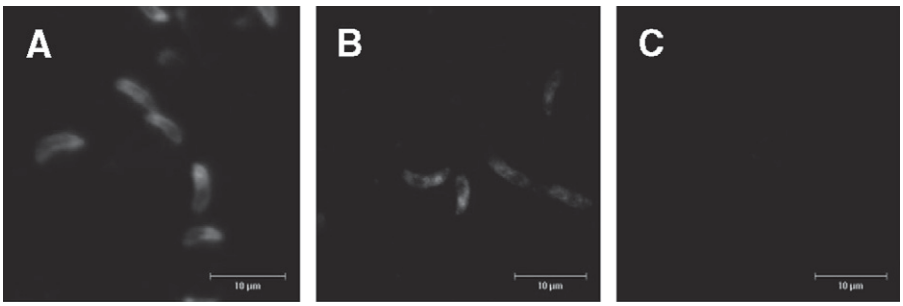

FIGURE 2. IFAT of human sera for reactivity against *N. caninum*. Slides were prepared with *N. caninum* tachyzoites as described and treated with a 1:100 human sera. **A**, Positive reactivity of serum from a mouse infected with *N. caninum* tachyzoites. **B**, Positive reactivity of human serum against *N. caninum* tachyzoites. **C**, Negative reactivity of human serum against *N. caninum* tachyzoites.

diagnostic candidates and were able to distinguish between *T. gondii* and *N. caninum* infection. More studies are needed to understand the high rates of these parasitic infections in Egypt. This study provides additional information of the prevalence of *T. gondii* and *N. caninum* infection in Delta, Egypt, and will assist in developing strategies for controlling the disease.

Received May 18, 2008. Accepted for publication September 25, 2008.

Acknowledgments: The authors thank J. P. Dubey (US Department of Agriculture, Agriculture Research Service, Livestock and Poultry Sciences Institute, and Parasite Biology and Epidemiology Laboratory) for supplying the *N. caninum* NC-1 isolate, local veterinary practitioners for collecting blood samples, and researchers at the Genetic Engineering and Biotechnology Institute (Minufiya University) who helped us during this work. This study was supported by the Egyptian Ministry of High Education and scientific research.

Authors' addresses: Hany M. Ibrahim, Penglong Huang, Xuenan Xuan, and Yoshifumi Nishikawa, Genetic Biochemistry Lab, National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan, Tel: 81-155-49-5886, Fax: 81-155-49-5643, E-mail: nisikawa@obihiro.ac.jp. Tarek A. Salem, Roba M. Talaat, and Mahmoud I. Nasr, Genetic Engineering and Biotechnology Institute, Minufiya University, Sadat City, PO Box 79, Egypt, Tel: 2-048-2601262, Fax: 2-048-2601268, E-mail: salem_tarek@yahoo.com.

REFERENCES

1. Dubey JP, Beattie CP, 1988. *Toxoplasmosis of Animals and Man*. Boca Raton, FL: CRC Press.
2. Tenter AM, Heckeroth AR, Weiss LM, 2000. *Toxoplasma gondii*: from animals to humans. *Int J Parasitol 30:* 1217–1258.
3. Chintana T, Sukthana Y, Bunyakai B, Lekkla A, 1998. *Toxoplasma gondii* antibody in pregnant women with and without HIV infection. *Southeast Asian J Trop Med Public Health 2*: 383–386.
4. Luft BJ, Remington JS, 1988. AIDS commentary. Toxoplasmic encephalitis. *J Infect Dis 157:* 1–6.
5. Frenkel JK, Escajadillo A, 1987. Cyst rupture as a pathogenic mechanism of toxoplasmic encephalitis. *Am J Trop Med Hyg 36:* 517–522.
6. Aspinall TV, Guy EC, Roberts KE, Joynson DH, Hyde JE, Sims PF, 2003. Molecular evidence for multiple *Toxoplasma gondii* infections in individual patients in England and Wales: public health implications. *Int J Parasitol 33:* 97–103.
7. Luft BJ, Remington JS, 1992. Toxoplasmic encephalitis in AIDS. *Clin Infect Dis 15:* 211–222.
8. Faria EB, Gennari SM, Pena HF, Athayde AC, Silva ML, Azevedo SS, 2007. Prevalence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in goats slaughtered in the public slaughterhouse of Patos city, Paraíba State, Northeast region of Brazil. *Vet Parasitol 149:* 126–129.
9. Anderson ML, Blanchard PC, Barr BC, Dubey JP, Hoffman RL, Conrad PA, 1991. *Neospora*-like protozoan infection as a major cause of abortion in California dairy cattle. *J Am Vet Med Assoc 198:* 241–244.
10. Barber JS, Trees AJ, 1996. Clinical aspects of 27 cases of neosporosis in dogs. *Vet Rec 139:* 439–443.
11. Dubey JP, 2003. Review of *Neospora caninum* and neosporosis in animals. *Korean J Parasitol 41:* 1–16.
12. Tranas J, Heinzen RA, Weiss LM, McAllister MM, 1999. Serological evidence of human infection with the protozoan *Neospora caninum. Clin Diagn Lab Immunol 5:* 765–767.
13. Lobato J, Silva DA, Mineo TW, Amaral JD, Segundo GR, Costa-Cruz JM, Ferreira MS, Borges AS, Mineo JR, 2006. Detection of immunoglobulin G antibodies to *Neospora caninum* in humans: high seropositivity rates in patients who are infected by human immunodeficiency virus or have neurological disorders. *Clin Vaccine Immunol 1:* 84–89.
14. Huang X, Xuan X, Hirata H, Yokoyama N, Xu L, Suzuki N, Igarashi I, 2004. Rapid immunochromatographic test using recombinant SAG2 for detection of antibodies against *Toxoplasma gondii* in cats. *J Clin Microbiol 42:* 351–353.
15. Jenkins M, Baszler T, Bjorkman C, Schares G, Williams D, 2002. Diagnosis and seroepidemiology of *Neospora caninum*-associated bovine abortion. *Int J Parasitol 32:* 631–636.
16. Chahan B, Gaturaga I, Huang X, Liao M, Fukumoto S, Hirata H, Nishikawa Y, Suzuki H, Sugimoto C, Nagasawa H, Fujizaki K, Igarashi I, Mikami T, Xuan X, 2003. Serodiagnosis of *Neospra caninum* infection in cattle by enzyme-linked immunosorbent assay with recombinant truncated NcSAG1. *Vet Parasitol 118:* 177–185.
17. Huang X, Xuan X, Kimbita EN, Battur B, Miyazawa T, Fukumoto S, Mishima M, Makala LH, Suzuki H, Sugimoto C, Nagasawa H, Fujisaki K, Mikami T, Igarashi I, 2002. Development and evaluation of an enzyme-linked immunosorbent assay with recombinant SAG2 for diagnosis of *Toxoplasma gondii* infection in cats. *J Parasitol 88:* 804–807.
18. Huang X, Xuan X, Suzuki H, Sugimoto C, Nagasawa H, Fujisaki K, Mikami T, Igarashi I, 2002. Characterization of *Toxoplasma gondii* SAG2 expressed in insect cells by recombinant baculovirus and evaluation of its diagnostic potential in an enzyme-linked immunosorbent assay. *Clin Diagn Lab Immunol 9:* 1343–1347.
19. Hemphill A, Fuchs N, Sonda S, Gottstein B, Hentrich B, 1997. Identification and partial characterization of a 36-kDa surface protein on *Neospora caninum* tachyzoites. *Parasitology 115:* 371–380.
20. Howe DK, Crawford AC, Lindsay D, Sibley LD, 1998. The p29 and p35 immunodominant antigens of *Neospora caninum* tachyzoites are homologous to the family of surface antigens of *Toxoplasma gondii. Infect Immun 66:* 5322–5328.
21. Azab ME, el-Shenawy SF, el-Hady HM, Ahmad MM, 1993. Comparative study of three tests (indirect haemagglutination, direct agglutination, and indirect immuno-fluorescence) for detection of antibodies to *Toxoplasma gondii* in pregnant women. *J Egypt Soc Parasitol 23:* 471–476.
22. El-Massey A, Mahdy OA, El-Ghaysh A, Dubey JP, 2000. Prevalence of *Toxoplasma gondii* antibodies from sera of turkeys, chickens, and ducks from Egypt. *J Parasitol 32:* 99–105.
23. Dubey JP, Graham DH, Dahl E, Hilali M, El-Ghaysh A, Sreekumar C, Kwok OC, Shen SK, Lehmann T, 2003. Isolation and molecular characterization of *Toxoplasma gondii* from chickens and ducks from Egypt. *Vet Parasitol 30:* 89–95.
24. El-Ghaysh A, 1998. Seroprevalence of *Toxoplasma gondii* in Egyptian donkeys using ELISA. *Vet Parasitol 15:* 71–73.
25. Hilali M, Romand S, Thulliez P, Kwok OC, Dubey JP, 1998. Prevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in sera from camels from Egypt. *Vet Parasitol 75:* 269–271.
26. 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 Egypt. *Int J Parasitol 3:* 527–529.
27. Dubey JP, Frenkel JK, 1988. Toxoplasmosis of rats: a review, with considerations of their value as an animal model and their possible role in epidemiology. *Vet Parasitol 77:* 1–32.
28. Parmley SF, Sgarlato GD, Mark J, Prince JB, Reminaton JS, 1992. Expression, characterization, and serological reactivity of recombinant surface antigen P22 of *Toxoplasma gondii. J Clin Microbiol 30:* 1127–1133.
29. Nimri L, Pelloux H, Elkhatib L, 2004. Detection of *Toxoplasma gondii* DNA and specific antibodies in high-risk pregnant women. *Am J Trop Med Hyg 71:* 831–835.
30. Remington JS, Mcleod R, Desmonts G, 1995. Toxoplasmosis. Remington JS, Klein JO, eds. *Infectious Diseases of the Fetus and Newborn Infant.* Philadelphia: W.B. Saunders, 140–267.
31. Abdel-Hafez SK, Shbeeb I, Ismail NS, Abdel-Rahman F, 1986. Serodiagnosis of *Toxoplasma gondii* in habitually aborting women and other adults from North Jordan. *Folia Parasitol (Praha) 33:* 7–13.
32. Galvan Ramirez ML, Soto Mancilla JL, Velasco Castrejon O, Perez Medina R, 1995. Incidence of anti-*Toxoplasma* antibodies

in women with high-risk pregnancy and habitual abortions. *Rev Soc Bras Med Trop 28:* 333–337.
33. Zuber P, Jacquier P, 1995. Epidemiology of toxoplasmosis: worldwide status. *Schweiz Med Wochenschr 65 (Suppl):* 19S–22S.
34. McAllister MM, Dubey JP, Lindsay DS, Jolley WR, Wills RA, McGuire AM, 1998. Dogs are definitive hosts of *Neospora caninum. Int J Parasitol 28:* 1473–1478.
35. McCabe RE, Remington JS, 1985. *Toxoplasma gondii*. Mandell GL, Douglas Jr RG, Bennett JE, eds. *Principles and Practice of Infectious Diseases.* Second edition. New York: John Wiley & Sons, 1540–1549.
36. Cook DC, Gilbert RE, Buffolano W, Zufferey J, Petersen E, Jenum PA, Foulon W, Semprini AE, Dunn DT, 2000. Sources of *Toxoplasma* infection in pregnant women: European multicentre case-control study. *BMJ 21:* 142–147.
37. Ghazy AA, Shaapan RM, Abdel-Rahman EH, 2007. Comparative serological diagnosis of toxoplasmosis in horses using locally isolated *Toxoplasma gondii. Vet Parasitol 145:* 31–36.