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Detection of *Neospora* from Tissues of Experimentally Infected Rhesus Macaques by PCR and Specific DNA Probe Hybridization

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***Neospora* is a newly recognized *Toxoplasma*-like cyst-forming coccidian parasite that causes abortion or congenital infections in naturally or experimentally infected animals. In this study, pregnant rhesus macaques were inoculated with culture-derived tachyzoites of a bovine *Neospora* isolate, and tissue samples from various major organs were collected from dams and fetuses for the detection of parasite DNA by using oligonucleotide primers COC-1 and COC-2 for PCR amplification of a conserved coccidial nuclear small-subunit rRNA gene sequence, and amplification products were confirmed by hybridization with a *Neospora*-specific DNA probe. PCR products were amplified from DNAs of different fetal monkey tissues, including brain, heart, lung, liver, spleen, skeletal muscle, skin, and placenta. In addition, *Neospora* DNA was amplified from the brain, heart, and lung tissues of infected rhesus macaque dams. The PCR and probe hybridization system may provide an effective method for the detection of *Neospora* infection in fetuses and dams from nonhuman primates and may be useful in determining the zoonotic potential of *Neospora*.**

A *Neospora* sp. was first identified as a *Toxoplasma*-like protozoan in dogs (10, 11, 21) and was subsequently recognized as a cause of bovine abortion (1, 5, 52) and neurological disease in calves (7, 8). Natural *Neospora caninum* infections have been reported in other domestic animals, including sheep (20, 24), horses (26), and goats (6, 18). Furthermore, *Neospora* infections have been produced experimentally in several animal species, including cattle (10, 25), sheep (24), and dogs (14). *Neospora* is morphologically similar to *Toxoplasma gondii* (8, 19), and the pathogeneses of the diseases produced by *Neospora* and *T. gondii* infections have many similarities (4, 13, 17). Recent studies based on nuclear small-subunit rRNA (nss-rRNA) gene sequence analysis further demonstrated that *N. caninum* and *T. gondii* are phylogenetically closely related (28, 31, 36, 44). Marsh et al. (44) showed that the nss-rRNA of the bovine *Neospora* isolate BPA-1 and *N. caninum* were homologous and differed from *T. gondii* at only 4 base pairs.

To date, there are no reports of natural *Neospora* infections in primate species. However, the susceptibility of nonhuman primates to neosporosis was confirmed by showing that experimental infection of pregnant rhesus macaques with a bovine *Neospora* isolate resulted in transplacental transmission and fetal infections comparable to those seen in human fetuses with *T. gondii* infection (10).

At present, a definitive diagnosis of clinical neosporosis can be established only by the identification of the parasites in postmortem tissue sections by immunohistochemical methods (1, 43). However, a PCR-based, molecular biology-based method for the detection of *Neospora* in blood and amniotic fluid samples from bovine and rhesus macaque fetuses has recently been reported (33). *Neospora* DNA was also detected in brain and/or lung tissues of an experimental murine model by using PCR primers derived from DNA sequences of the *N. caninum* 14-3-3 gene (42), internal transcribed spacer 1 of the rRNA gene (37), and a cloned *Neospora*-specific genomic DNA fragment (57). Nevertheless, the organ distribution of *Neospora* in other experimentally infected animals has not been evaluated by PCR or specific probe detection techniques. A newly developed PCR-based amplification and hybridization system with a specific probe (33) was used in this study to detect and evaluate the *Neospora* parasite distribution in different tissue samples of experimentally infected rhesus macaque dams and fetuses.

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MATERIALS AND METHODS

Parasite cultivation and DNA isolation. *Neospora* sp. isolate BPA-1, obtained from an aborted bovine fetus (15), and *T. gondii* RH (kindly provided by John Boothroyd, Stanford University, Palo Alto, Calif.), isolated from a child with toxoplasmosis (47), were propagated continuously in vitro in stationary-phase monolayer cultures of bovine cardiopulmonary aortic endothelial (CPAE) cells (ATCC CCL209) as described previously (15). Tachyzoites were harvested when ≥80% of the monolayer cell cultures were infected. The cell monolayers were removed by scraping, and the resuspended cells in medium were passed three times through a 25-gauge needle, filtered through a 5-μm-pore-size filter, centrifuged at 1,200 × *g* for 10 min, and then resuspended in 1 ml of Dulbecco's minimum essential medium (GIBCO/BRL, Grand Island, N.Y.). The numbers of tachyzoites were determined by counting culture aliquots diluted with phosphate-buffered saline (PBS; pH 7.4) in a hemocytometer by using a light microscope (×200 magnification). Purified DNA was prepared from tachyzoites (10^6) that had been washed once with PBS and resuspended in 100 μl of sample buffer solution provided with the IsoQuick Nucleic Acid Extraction kit (ORCA Research Inc., Bothell, Wash.). The tachyzoite suspension was mixed with 100 μl of lysis solution, 700 μl of extraction matrix, and 400 μl of extraction buffer, vortexed for 10 s, and centrifuged at 12,000 × *g* for 5 min. The aqueous phase was removed and mixed with an equal volume of ice-cold isopropanol and a 0.1 volume of sodium acetate to precipitate the DNA by placing the mixture at −20°C for 2 h or overnight. The DNA pellet obtained after centrifugation (12,000 × *g* for 10 min at 4°C) was rinsed with 70% (vol/vol) ethanol, air dried, and dissolved in RNase-free water (ORCA Research Inc.) for PCR amplification. The same procedure was used to prepare control DNA from uninfected CPAE monolayer cells that were scraped from flasks, pelleted at 1,300 × *g* for 10 min, and washed once with PBS. The supernatant was removed, and the unin-

fected CPAE cell pellet was stored at −20°C until it was used as described previously (44).

Experimental infections. In this study two experimentally infected pregnant macaques (*Macaca mulatta*) (animals 2a and 2b) and one control pregnant rhesus macaque (animal 2c) were housed separately and were monitored according to the standards of the Federal Animal Welfare Act and the Institute for Laboratory Animal Resources, as described previously (9). Briefly, all female monkeys were bred midcycle, and pregnancy was confirmed preinoculation by ultrasound examination (51). Two pregnant monkeys (animals 2a and 2b) were inoculated intramuscularly and intravenously on gestational day (GD) 43 with a total of 1.6×10^7 in vitro-cultivated *Neospora* (isolate BPA-1) tachyzoites (15) in order to elicit fetal infection near the start of the second trimester. The pregnant control monkey (animal 2c) was inoculated on the same GD, 43, with uninfected culture material. Fetuses were sonographically monitored on the day when *Neospora* tachyzoites or uninfected vehicle were administered, then every 2 days for 2 weeks, and weekly thereafter (51). Viability, growth, and development were assessed at each examination. The fetuses were recovered by hysterotomy between 67 and 70 days postinoculation, and the dams were subsequently euthanized. Maternal and fetal tissues were collected for PCR amplification and regular histopathological examination. Fetal tissue samples were also processed for in vitro parasite isolation, and immunohistochemical examination was performed as described previously (9) to detect and locate parasites in tissue samples.

Preparation of DNA from monkey tissues for PCR. Monkey dam and fetal tissues were collected during necropsy by using alcohol-flamed scissors and forceps for each piece of tissue to prevent cross-contamination. All tissues were frozen at −20°C until DNA isolation was performed. DNA was isolated from monkey tissues with a Mini-beadbeater machine (Biospec Products, Bartlesville, Okla.) and chemical reagents in the IsoQuick Nucleic Acid Extraction kit (ORCA Research Inc.). Briefly, frozen tissues were thawed and approximately 0.1 g of tissues was added to 2-ml screw-cap vials filled to 85% in volume with 1.0-mm-diameter sterile glass beads (Biospec Products). An equal volume of sample buffer and lysis solution (ORCA Research Inc.) containing guanidine thiocyanate was added to fill the remaining volumes of the vials. The caps of the vials were tightly screwed onto the vials, and the vials were inserted into the arm assembly of the Mini-beadbeater and vibrated at 5,000 rpm for 5 to 10 min. Then the vials were removed and the top and bottom portions of the vials were carefully sliced open with a sterile razor blade. Tissue lysate was removed from the vials by centrifugation at $400 \times g$ for 3 min. The tissue DNA was isolated with the IsoQuick Nucleic Acid Extraction kit, following the manufacturer's protocol.

Evaluation of spiked tissue samples. To test for the presence of substances in monkey tissues inhibitory to PCR and evaluate the specificity of the *Neospora* and *Toxoplasma* internal probes, 50 in vitro-cultivated *Neospora* tachyzoites or 25 *T. gondii* tachyzoites were mixed with approximately 0.1 g of the different tissues from the uninfected control rhesus macaques, dam 2c or fetus 2c. DNA was isolated with the IsoQuick Nucleic Acid Extraction kit as described above.

PCR primers and specific internal probes. Oligonucleotide primers for PCR (COC-1 [5′-AAGTATAAGCTTTTATACGGCT-3′] and COC-2 [5′-CACTGC CACGGTAGTCCAATAC-3′]) were designed by comparing the nss-rRNA gene sequences of bovine *Neospora* isolates to those of *N. caninum*, *T. gondii*, *Sarcocystis* spp., and *Cryptosporidium parvum* as described previously (44). Four nucleotide variations located at nucleotides 167, 222, 254, and 689 were consistently detected when sequences of *Neospora* isolates were compared to the *T. gondii* sequence (44). Probes specific for a *Neospora* sp. (5′-AGTCAAACGCG-3′) and *T. gondii* (5′-AAGTCAACGCG-3′) were selected on the basis of the nucleotide at position 254, which is a thymine base in *Neospora* isolates but is absent from *T. gondii.*

PCR amplification and Southern blot hybridization. DNA amplification was carried out in a total volume of 50 μl by using approximately 50 to 100 ng of DNA mixed with 10 mM Tris-HCl (pH 9.0), 50 mM potassium chloride, 0.1% Triton X-100, 1.0 mM magnesium chloride, 200 μM (each) deoxynucleoside triphosphate, 0.41 μM bovine *Neospora* PCR primer (COC-1), 0.40 μM bovine *Neospora* PCR primer (COC-2), and 2.5 U of *Taq* DNA polymerase (Promega Corp., Madison, Wis.). Amplification was carried out in HotStart 50 Storage and Reaction Tubes (Molecular Bio-Products Inc., San Diego, Calif.) and was performed in a DNA thermal cycler (Perkin-Elmer Cetus Corp., Norwalk, Conn.) for 51 cycles, with each cycle consisting of denaturation at 94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 2 min. The last cycle was given a prolonged extension period of 7 min. Negative controls containing the reagents but no DNA were included with each batch of test samples. To avoid carryover of contaminating nucleic acids which may result in false-positive reactions (41, 45), the steps of pre-PCR sample preparation, mixing of PCR reagents with the samples, and the final PCR thermal cycles were performed in separate laboratories.

After amplification, 5 μl of each sample or the BioMarker Low DNA size standard (BioVentures, Inc., Murfreesboro, Tenn.) was electrophoresed on a 3% Nusieve 3:1 agarose gel (FMC Bioproducts, Rockland, Maine) that had been stained with 0.5 μg of ethidium bromide per ml and was examined under UV illumination. Amplification products were transferred to nylon membranes (Hybond-N^+; Amersham Corp., Arlington Heights, Ill.) by the Southern blotting method (48) and were cross-linked with a Stratalinker UV crosslinker (Stratagene, La Jolla, Calif.). Prehybridization, preparation of the labeled internal probe, and hybridization were performed as recommended by the manufacturer for the Enhanced Chemiluminescence 3′-Oligolabeling and Detection Systems (Amersham Corp.). Labeled internal probe (*Neospora* specific or *Toxoplasma* specific) was added to a final concentration of 10 ng/ml of hybridization solution, and the solution was incubated overnight at 30°C with gentle agitation. After hybridization, the membranes were washed twice for 5 min each time at room temperature in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–sodium dodecyl sulfate (SDS) and then twice for 5 min each time at room temperature in 0.5× SSC–0.1% (wt/vol) SDS. These levels of hybridization and stringency were critical to ensuring that the probes did not show cross-reactivity between the *T. gondii* and *Neospora* amplification products. Membrane blocking, antibody incubations, signal generation, and signal detection were performed as described by the manufacturer. Membranes were exposed to Kodak X-Omat film for 3 to 10 min.

RESULTS

Evaluation of spiked tissue samples. By using primers COC-1 and COC-2, PCR products of 294 bp were amplified from DNA samples of all the different types of uninfected rhesus macaque tissue samples (dam or fetus 2c) that were spiked with *Neospora* or *T. gondii* tachyzoites. PCR inhibitors were not detected in the tested fetal monkey tissues (fetus 2c), including brain, heart, lung, liver, spleen, kidney, skeletal muscle, skin, and placenta, that were spiked with *Neospora* (Fig. 1A, lanes 6 to 14) or *T. gondii* (Fig. 1B, lanes 6 to 14). No amplification products were produced from CPAE monolayer cells or negative controls with reagents only in repeated experiments (Fig. 1A and B, lanes 4 and 5). All samples were retested at least three times on separate occasions to confirm consistent results. Only the amplification products produced with DNAs of culture-derived *Neospora* (BPA-1 isolate) tachyzoites (Fig. 1C and D, lane 2) and control monkey (fetus 2c) tissues spiked with *Neospora* tachyzoites (Fig. 1C, lanes 6 to 14) showed hybridization signals with the *Neospora*-specific chemiluminescent internal probe, whereas the *Toxoplasma*-specific internal probe hybridized to PCR products from DNAs of *T. gondii* RH tachyzoites (Fig. 1E and F, lanes 3) and tissue samples spiked with *T. gondii* (Fig. 1F, lanes 6 to 14) only. For monkey dam 2c, results identical to those for fetus 2c in the spiking experiment were obtained by using the same type of tissue samples, except that placenta was replaced with uterus, for PCR amplification and probe hybridization (data not shown).

Detection of *Neospora* in monkey samples. Figure 2 shows the PCR amplification and hybridization results from an evaluation of a variety of tissue samples from monkey fetus 2a transplacentally infected with *Neospora*. Amplification products were obtained from brain, heart, lung, spleen, skin, and placenta samples (Fig. 2A, lanes 6, 7, 8, 10, 13, and 14, respectively) from this monkey fetus in at least one of three PCR amplification tests (summarized in Table 1). The *Neospora*-specific internal probe hybridized to PCR products from all of the monkey tissues tested (Fig. 2B, lanes 6, 7, 8, 11, 13, and 14, respectively), but none of the amplification products hybridized to the *Toxoplasma*-specific probe.

In the same way, DNAs from samples of different tissues from the monkey dams and fetuses were used to evaluate the PCR and probe hybridization system in detecting *Neospora* infection. All monkey samples were tested three times on separate occasions, each time using a different piece of the tissue sample for PCR amplification. PCR products were amplified from DNAs of different tissues, including brain, heart, lung, liver, spleen, skeletal muscle, skin, and placental samples of monkey fetuses 2a and 2b (Table 1). In addition, positive PCR and probe hybridization results were obtained from the brain, heart, and lung tissues of monkey dams 2a and 2b (Table 1). Control monkey 2c and fetus 2c were inoculated with unin-

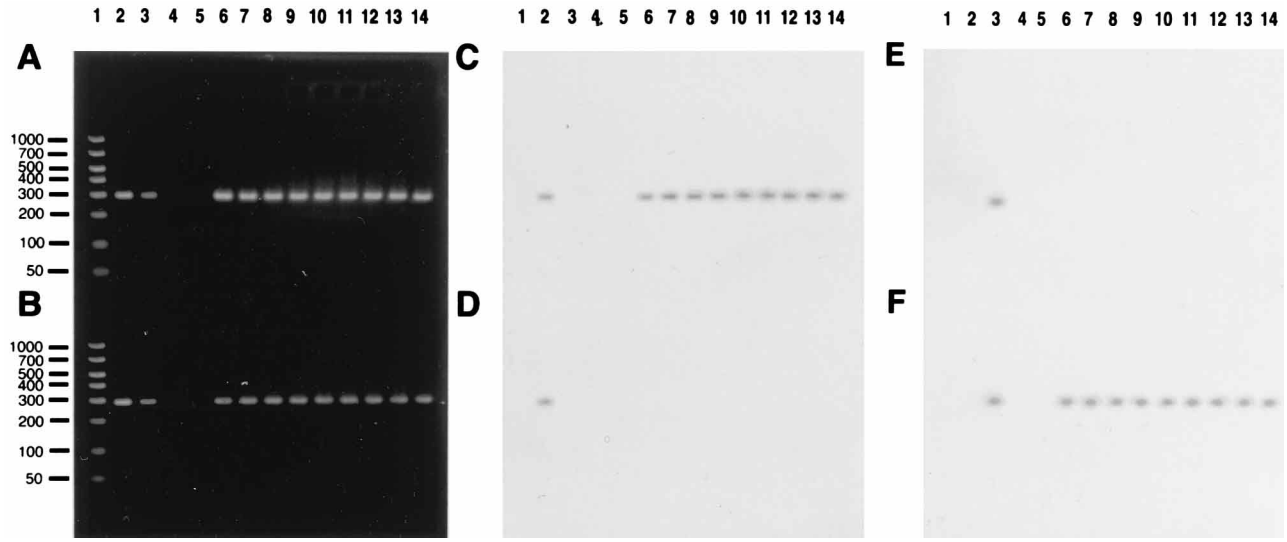

FIG. 1. Detection of *Neospora* and *T. gondii* by PCR amplification of uninfected monkey tissue samples spiked with tachyzoites. (A and B) Amplification products were observed under UV light after electrophoresis in an ethidium bromide-stained 3% Nusieve 3:1 agarose gel. Southern blots of the same gel hybridized to *Neospora*-specific (C and D) or *Toxoplasma*-specific (E and F) chemiluminescent internal probes are also shown. (A to F) Lane 1, BioMarker Low DNA size standard (base pair designations appear on the left); lane 2, a bovine *Neospora* sp. (BPA-1 isolate) DNA control; lane 3, *T. gondii* RH DNA control; lane 4, CPAE monolayer cell DNA control; lane 5, negative control with reagents only. (A, C, and E) Lanes 6 to 14, 50 *Neospora* tachyzoites added to samples from uninfected control fetus 2c consisting of brain, heart, lung, liver, spleen, kidney, skeletal muscle, skin, and placenta, respectively. (B, D, and F) Lanes 6 to 14, 25 *T. gondii* tachyzoites added to samples from uninfected control fetus 2c consisting of brain, heart, lung, liver, spleen, kidney, skeletal muscle, skin, and placenta, respectively.

fected monolayer cell culture, and no *Neospora* DNA was detected in samples from these animals.

DISCUSSION

Recently, we described the development of the COC-1 and COC-2 primers for PCR amplification and use of a *Neospora*-specific oligonucleotide probe to detect *Neospora* parasites in whole blood and amniotic fluid samples from experimentally infected rhesus macaque fetuses (33). In the present investigation we used the same system to confirm the identities of the PCR products produced from different fetal and maternal tissue samples of nonhuman primates. Our previous report showed that *Neospora* tachyzoites were detected by immunohistochemistry in the brain and amnion tissues of monkey fetuses 2a and 2b (9) but not in tissue samples from monkey dams 2a and 2b (unpublished data). *Neospora* parasites are most frequently identified by histopathological staining and/or immunohistochemistry with *Neospora* antisera in the fetal brain and spinal cord samples from naturally infected dogs and

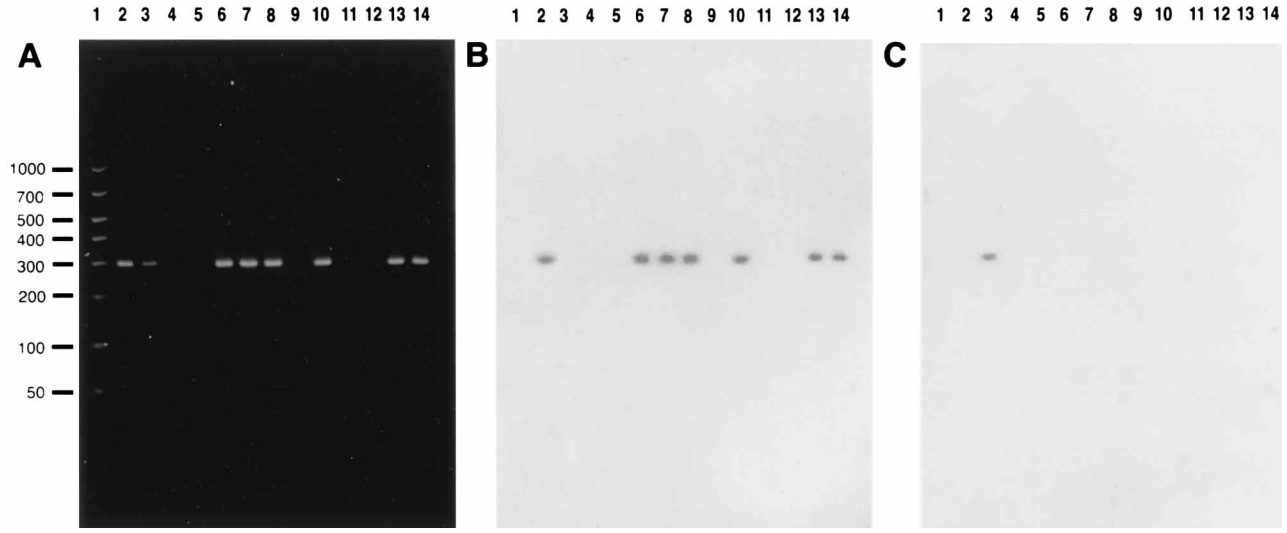

FIG. 2. Detection of *Neospora* by PCR amplification of monkey tissue samples from transplacentally infected fetus 2a. (A) Amplification products were observed under UV light after electrophoresis in an ethidium bromide-stained 3% Nusieve 3:1 agarose gel. Southern blots of the same gel hybridized to *Neospora*-specific (B) or *Toxoplasma*-specific (C) chemiluminescent internal probes are also shown. (A to C) Lane 1, BioMarker Low DNA size standard (base pair designations appear on the left); lane 2, a bovine *Neospora* sp. (BPA-1 isolate) DNA control; lane 3, *T. gondii* RH DNA control; lane 4, CPAE monolayer cell DNA control; lane 5, negative control with reagents only; lanes 6 to 14, samples from transplacentally infected fetus 2a consisting of brain, heart, lung, liver, spleen, kidney, skeletal muscle, skin, and placenta, respectively.

TABLE 1. PCR and probe hybridization results for the detection of *Neospora* from experimentally infected rhesus macaques

Tissue	PCR and probe hybridization result[a]																	
	Dam									Fetus								
	2a			2b			2c			2a			2b			2c		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
Brain	−	−	+	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−
Heart	−	−	−	−	+	−	−	−	−	+	−	+	−	−	+	−	−	−
Lung	+	−	−	−	−	−	−	−	−	+	+	−	+	−	−	−	−	−
Liver	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−
Spleen	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−
Kidney	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Skeletal muscle	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Skin	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−
Uterus	−	−	−	−	−	−	−	−	−	NA	NA	NA	NA	NA	NA	NA	NA	NA
Placenta	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	+	+	+	+	+	−	−	−

[a] Pregnant dams 2a and 2b were inoculated intramuscularly and intravenously on GD 43 with a total of 1.6×10^7 culture-derived *Neospora* tachyzoites. Pregnant control dam 2c was given an inoculum consisting of the uninfected monolayer cell cultures. Fetuses 2a, 2b, and 2c were removed by hysterotomy between 67 and 70 days postinoculation. All monkey samples were tested three times on separate occasions, and each time they were tested with a different piece of tissue sample for the PCR amplification assay. −, no amplification product; +, amplification of *Neospora* DNA confirmed by probe hybridization; NA, not applicable.

cattle (8, 16, 22, 38). However, *Neospora* tachyzoites and/or cysts have also been identified in a variety of bovine or canine tissue samples including heart (23, 39), lung (30, 39, 56), liver (39), muscle (16, 53), and placenta (56). In this study, parasites were not detected in the kidney or eye, as reported for cattle (52) and dogs (22), respectively. However, *Neospora* DNA was amplified from spleen and skin tissues from infected monkey fetuses. In addition, using PCR technology we were able to detect *Neospora* DNA in many other tissue samples (Table 1).

Neospora DNA was detected consistently in the central nervous system (CNS) of the infected monkeys and sporadically in a variety of non-CNS tissues. This pattern of detection of *Neospora* in non-CNS tissues probably is a reflection of both the sensitivity limits of the PCR-probe detection method, suggesting that only very small quantities of *Neospora* parasites are present in these non-CNS tissues, and the sporadic distribution of *Neospora* parasites at any given time in these non-CNS tissues, reflecting the pathogenesis of the disease. It seems likely, for instance, that this sporadic and inconsistent distribution in non-CNS tissues may be a manifestation of a constant blood-borne dissemination of small numbers of *Neospora* parasites. This hypothesis would be consistent with the sporadic detection of *Neospora* parasites in the blood of infected animals (33) and with the known pathogenesis of parasite dissemination with other related parasites such as *T. gondii* (32, 50). Given a constant low-grade shedding and dissemination of *Neospora* tachyzoites through the blood, these parasites could then randomly infect endothelial cells (4) throughout the body with subsequent parenchymal invasion in those organs where *Neospora* has a particular tropism. The relatively high percentage of detection in the lung (Table 1), where there is a particularly high volume of blood capillaries, would support this contention. This proposed mechanism of constant but sporadic shedding of small numbers of parasites through the blood of chronically infected animals would also indicate the futility in trying to develop an accurate antemortem diagnostic blood test that is based on the detection of either *Neospora* DNA or non-secreted *Neospora* antigens. Furthermore, it would suggest that in the chronically infected dam the uterus is continually exposed to small numbers of *Neospora* tachyzoites, a first step in understanding the pathogenesis of repeat fetal infections. However, the results also suggest that while the PCR-probe test is not optimal for the detection of antemortem infection in the blood of infected animals, it is far more sensitive than standard histology or immunohistochemistry in detecting the distribution of *Neospora* infection in tissues and is therefore a useful tool in disease pathogenesis studies.

Old World nonhuman primates are relatively resistant to both clinical disease (27) and transplacental fetal infection (55) following experimental inoculation with *T. gondii*, limiting the use of these species as animal models of human toxoplasmosis. In particular, it has not been possible to consistently produce transplacental fetal toxoplasmosis in rhesus monkeys, and when successful, the resultant fetal infections appear to have been subclinical in nature (49, 55). In contrast, the results from this study, in conjunction with results reported earlier (9), indicate that experimental neosporosis in the Old World primate may be a useful model for studying the pathogenesis of apicomplexa parasitic pathogens, including toxoplasmosis. To date, very little is known about the detailed pathogenesis of chronic *T. gondii* infection, its immunopathogenesis, and the mechanisms of transplacental infection in the primate hemochorial placenta. Much of our understanding of human fetal and congenital toxoplasmosis is based solely on traditional anatomical pathology and retrospective analysis of human cases of infection (29, 46). Because of the close relationship of the antigenic, morphologic, and biological nature of *Neospora* to *T. gondii*, nonhuman primate infectivity studies with *Neospora* would be of great assistance in providing valuable answers to questions regarding disease mechanism and host-parasite interactions unique to primate species including humans.

In this study we have shown that the PCR-probe test is a highly sensitive means of detecting small quantities of *Neospora* DNA in both fetal and maternal tissues. In particular, *Neospora*-specific PCR products were amplified from the brains, hearts, and lungs of infected rhesus macaque dams, which indicated that *Neospora* infections persist for at least 67 to 70 days postinoculation. Interestingly, *T. gondii* DNA was amplified by PCR most reliably from brain, heart, and lung lavage samples from patients with immunological disorders and disseminated toxoplasmosis (12, 34, 35, 40, 54, 55) similar to both naturally acquired *T. gondii* and *Neospora* infections. However, it remains to be seen whether the maternal *Neospora* infection would persist through subsequent pregnancies result-

ing in repeat fetal infection, as occurs with persistent *Neospora* infections in cattle and dogs (3, 22). Further studies are necessary to determine whether maternal infection persists and, if so, whether repeat transplacental fetal infection can occur in the nonhuman primate, as has been documented for other animal species (2, 7, 22). Additional studies on the immunopathogenesis of *Neospora* infection in nonhuman primates with chronic infections versus the immunopathogenesis in those with acute infections, the specific trafficking of parasites at the maternal-fetal interface leading to fetal infection, the outcome of fetal encephalitis, and the possible production of congenital infections mimicking human congenital toxoplasmosis could provide vital information for obtaining a better understanding of both human toxoplasmosis and neosporosis.

To date, no human cases of neosporosis have been reported. However, the biological and molecular similarities between *Neospora* and *T. gondii* and the susceptibility of rhesus macaques to experimental infection with *Neospora* tachyzoites (9) suggest that the zoonotic potential of *Neospora* warrants consideration. The PCR and specific probe hybridization system described here is a valuable tool for the detection of *Neospora* infection in nonhuman primate fetuses and dams. This methodology may also prove useful for determining if such infections occur in human patients with *Toxoplasma*-like disease.

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