Prevalence of Antibodies to *Trypanosoma cruzi* in Raccoons (*Procyon lotor*) From an Urban Area of Northern Virginia

Author(s): Katie Hancock , Anne M. Zajac , Oscar J. Pung , Francois Elvinger , Alexa C. Rosypal , and David S. Lindsay

Source: Journal of Parasitology, 91(2):470-472. 2005.
Published By: American Society of Parasitologists
DOI: http://dx.doi.org/10.1645/GE-399R
URL: http://www.bioone.org/doi/full/10.1645/GE-399R

Foundation and the BK21 Human Life Sciences, Ministry of Education, Republic of Korea.

LITERATURE CITED

CANDOLFI, E., C. A. HUNTER, AND J. S. REMINGTON. 1994. Mitogen- and antigen-specific proliferation of T cells in murine toxoplasmosis is inhibited by reactive nitrogen intermediates. Infection and Immunity **62:** 1995–2001.

———, ———, AND ———. 1995. Roles of gamma interferon and other cytokines in suppression of the spleen cell proliferative response to concanavalin A and *Toxoplasma* antigen during acute toxoplasmosis. Infection and Immunity **63:** 751–756.

CHAI, J. Y., J. KOOK, S. M. GUK, Y. P. CHANG, AND C. K. YUN. 1997. Experimental infection of murine splenic lymphocytes and granulocytes with *Toxoplasma gondii* RH tachyzoites. Korean Journal of Parasitology **35:** 79–85.

CHANNON, J. Y., AND L. H. KASPER. 1996. *Toxoplasma gondii*–induced immune suppression by human peripheral blood monocytes: Role of gamma interferon. Infection and Immunity **64:** 1181–1189.

———, E. I. SUH, R. M. SEGUIN, AND L. H. KASPER. 1999. Attachment ligands of viable *Toxoplasma gondii* induce soluble immunosuppressive factors in human monocytes. Infection and Immunity **67:** 2547–2551.

HAQUE, S., H. DUMON, A. HAQUE, AND L. H. KASPER. 1998. Alteration of intracellular calcium flux and impairment of nuclear factor-AT translocation in T cells during acute *Toxoplasma gondii* infection in mice. Journal of Immunology **161:** 6812–6818.

———, A. HAQUE, AND L. H. KASPER. 1995. A *Toxoplasma gondii*–derived factor(s) stimulates immune downregulation: An in vitro model. Infection and Immunity **63:** 3442–3447.

———, I. KHAN, A. HAQUE, AND L. KASPER. 1994. Impairment of the cellular immune response in acute murine toxoplasmosis: Regulation of interleukin 2 production and macrophage-mediated inhibitory effects. Infection and Immunity **62:** 2908–2916.

HIRAMOTO, R. M., A. J. GALISTEO, JR., N. D. NASCIMENTO, AND H. F. DE ANDRADE, JR. 2002. 200 Gy sterilised *Toxoplasma gondii* tachyzoites maintain metabolic functions and mammalian cell invasion, eliciting cellular immunity and cytokine response similar to natural infection in mice. Vaccine **20:** 2072–2081.

KHAN, I. A., T. MATSUURA, AND L. H. KASPER. 1995. IL-10 mediates immunosuppression following primary infection with *Toxoplasma gondii* in mice. Parasite Immunology **17:** 185–195.

KOOK, J., Y. J. KIM, M. SEO, AND J. Y. CHAI. 1995. TEM ultrastructure of gamma-irradiated *Toxoplasma gondii* RH tachyzoites. Seoul Journal of Medicine **36:** 159–165.

MUN, H. S., F. AOSAI, M. CHEN, L. X. PIAO, K. NOROSE, Y. IWAKURA, AND A. YANO. 2003. Pathogenicity of *Toxoplasma gondii* through B-2 cell-mediated downregulation of host defense responses. Microbiology and Immunology **47:** 533–542.

NASH, P. B., M. B. PURNER, R. P. LEON, P. CLARKE, R. C. DUKE, AND T. J. CURIEL. 1998. *Toxoplasma gondii*–infected cells are resistant to multiple inducers of apoptosis. Journal of Immunology **160:** 1824–1830.

NEYER, L. E., H. KANG, J. S. REMINGTON, AND Y. SUZUKI. 1998. Mesenteric lymph node T cells but not splenic T cells maintain their proliferative response to concanavalin A following peroral infection with *Toxoplasma gondii*. Parasite Immunology **20:** 573–581.

SHIN, E. H., S. B. KIM, H. W. NAM, E. T. HAN, J. H. PARK, H. J. AHN, AND J. Y. CHAI. 2004. Use of monoclonal antibodies for flow cytometric detection of intracellular *Toxoplasma gondii* in murine splenic lymphocytes. Journal of Parasitology **90:** 161–166.

SUZUKI, Y., AND A. KOBAYASHI. 1983. Suppression of unprimed T and B cells in antibody responses by irradiation-resistant and plastic-adherent suppressor cells in *Toxoplasma gondii*-infected mice. Infection and Immunity **40:** 1–7.

———, M. A. ORELLANA, R. D. SCHREIBER, AND J. S. REMINGTON. 1988. Interferon-γ: The major mediator of resistance against *Toxoplasma gondii*. Science **240:** 516–518.

———, N. WATANABE, AND A. KOBAYASHI. 1981. Nonspecific suppression of primary antibody responses and presence of plastic-adherent suppressor cells in *Toxoplasma gondii*–infected mice. Infection and Immunity **34:** 30–35.

VAN OVERTVELT, L., N. VANDERHEYDE, V. VERHASSELT, J. ISMAILI, L. DE VOS, M. GOLDMAN, F. WILLEMS, AND B. VRAY. 1999. *Trypanosoma cruzi* infects human dendritic cells and prevents their maturation: Inhibition of cytokines, HLA-DR, and costimulatory molecules. Infection and Immunity **67:** 4033–4040.

WEI, S., F. MARCHES, J. BORVAK, W. ZOU, J. CHANNON, M. WHITE, J. RADKE, M. F. CESBRON-DELAUW, AND T. J. CURIEL. 2002. *Toxoplasma gondii*–infected human myeloid dendritic cells induce T-lymphocyte dysfunction and contact-dependent apoptosis. Infection and Immunity **70:** 1750–1760.

J. Parasitol., 91(2), 2005, pp. 470–472

Prevalence of Antibodies to *Trypanosoma cruzi* in Raccoons (*Procyon lotor*) From an Urban Area of Northern Virginia

Katie Hancock, Anne M. Zajac, Oscar J. Pung*, Francois Elvinger†, Alexa C. Rosypal, and David S. Lindsay‡, Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, 1410 Prices Fork Road, Blacksburg, Virginia 24061-0342; *Department of Biology, Georgia Southern University, Statesboro, Georgia 30460-8042; †Department of Large Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, 1410 Prices Fork Road, Blacksburg, Virginia 24061-0342; ‡To whom correspondence should be addressed. *e-mail: lindsayd@vt.edu*

ABSTRACT: Raccoons (*Procyon lotor*) are reservoir hosts for *Trypanosoma cruzi*. A 3-yr-long serological survey was conducted to determine the prevalence of antibodies to this zoonotic parasite in raccoons collected from Fairfax County, Virginia, a suburban/urban area outside Washington, D.C. Serum samples from 464 raccoons were examined for *T. cruzi* antibodies at a 1:40 dilution with an indirect fluorescent antibody test (IFAT) and Brazil strain *T. cruzi* amastigotes and trypomastigotes as antigen. A positive IFAT test was found in 154 (33%) of the 464 samples. The yearly prevalence was 49 of 132 (37%) in 2000; 19 of 120 (16%) in 2001; and 86 of 212 (41%) in 2002. Our study indicates that raccoons in this area of Virginia are frequently exposed to *T. cruzi*.

This study was conducted to determine the serological prevalence of antibodies to *Trypanosoma cruzi* in a common reservoir host, the raccoon (*Procyon lotor*), from Virginia. Our interest was in part a result of the report of clinical *T. cruzi* infection (Barr et al., 1995) in dogs (Walker hounds) from Virginia. Schaffer et al. (1978) indicated that none of 10 raccoons from Virginia were blood culture–positive for *T. cruzi*. Raccoons used in the present study originated in various locations in Fairfax County, Virginia, a suburban/urban area outside Washington, D.C. Raccoons used for this study were live-trapped as part of a larger study on rabies. Blood samples were collected from all trapped raccoons. Raccoons were released immediately after sampling was completed. The serum was collected, placed in a tube, and frozen at −70

TABLE I. Prevalence of *Trypanosoma cruzi* in raccoons from the United States.

Location	No. examined/ No. positive (% positive)	Test used*	Reference
Florida	184/2 (1%)	BS	Telford and Forrester, 1991
Florida	33/4 (12%)	Culture	Schaffer et al., 1978
Florida/Georgia	608/9 (1.5%)	Culture	McKeever et al., 1958
Georgia	10/5 (50%)	Culture	Schaffer et al., 1978
Georgia	54/12 (22%)	Culture	Pung et al., 1995
Georgia	30/13 (43%)	Culture	Pietrzak and Pung, 1998
Georgia	87/51 (59%)	IFAT	Yabsley and Noblet, 2002
Maryland	400/5 (1%)	Culture	Walton et al., 1958
North Carolina	20/3 (15%)	Culture	Karsten et al., 1992
Oklahoma	8/5 (63%)	Culture	John and Hoppe, 1986
South Carolina	134/53 (40%)	IFAT	Yabsley and Noblet, 2002
Tennessee	6/0 (0%)	Culture	Schaffer et al., 1978
Tennessee	3/2 (67%)	Culture	Herwaldt et al., 2000
Texas	25/6 (24%)	Culture	Schaffer et al., 1978
Virginia	10/0 (0%)	Culture	Schaffer et al., 1978
Virginia	464/154 (33%)	IFAT	Present study
West Virginia	10/0 (0%)	Culture	Schaffer et al., 1978

* BS = blood smear, Culture = blood culture; IFAT = indirect fluorescent antibody test.

C. Frozen sera were sent to the Center for Molecular Medicine and Infectious Diseases (Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia) for testing.

The indirect fluorescent antibody test (IFAT) was used because it has been shown to be more sensitive than blood culture in detecting *T. cruzi* infections in raccoons (Yabsley et al., 2001). Brazil strain *T. cruzi* amastigotes and trypomastigotes were collected from bovine monocyte cell cultures. Parasites were washed in phosphate-buffered saline (PBS) by centrifugation, air-dried onto 12-well microscope slides, fixed in 100% methanol for 30 sec, and stored at −20 C until used. Sera were diluted to 1:40 in PBS and incubated with *T. cruzi* antigens for 30 min at room temperature. The slides were then washed 2 times in PBS for 5 min per washing. Bound antibodies were detected by incubation with goat anti-raccoon IgG(H&L) (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland) at a 1:40 dilution in PBS for 30 min at room temperature. The slides were then washed 2 times in PBS for 5 min per washing. All incubations were done in a humidified box. The IFAT slides were mounted in fluoromount-G (Southern Biotechnology Associates, Birmingham, Alabama) and covered with cover slips (24 × 60 mm) and viewed with an Olympus BX60 fluorescent microscope. Sera from *T. cruzi* blood culture–negative and blood culture–positive raccoons (Pung et al., 1995) were used as controls on each IFAT slide.

Serological examination by IFAT found that 154 of the 464 (33%) raccoons sampled during the 3-yr period were positive. The yearly prevalence was 49 of 132 (37%) in 2000; 19 of 120 (16%) in 2001; and 86 of 212 (41%) in 2002.

Trypanosoma cruzi has been isolated from raccoons from as far north as Maryland (Walton et al., 1958). Infectivity of these isolates from Maryland for mammals (Diamond and Rubin, 1958; Walton et al., 1958) and triatomid insects (Walton et al., 1958) has been demonstrated. The prevalence of infection in raccoons from Maryland was 1% (5 of 400), which is lower than the 33% (154 of 464) for raccoons from Virginia in this study. Our prevalence results are similar to those reported by others for raccoons in the southern United States (Table I) when IFAT or culture are used to conduct examinations. Schaffer et al. (1978) did not isolate *T. cruzi* from the 10 raccoons they examined from Virginia.

Clinical canine *T. cruzi* infections have been reported in dogs from Virginia (Barr et al., 1995). We are not aware of autochthonous human infections acquired in Virginia. There are apparently only 5 cases of autochthonous vectorborne human *T. cruzi* infections reported in the United States (see Herwaldt et al., 2000). Three cases were in children <10 mo old from Texas, 1 case was in an 18-mo-old child from Tennessee, and 1 case was in a 56-yr-old adult from California (see Herwaldt et al., 2000).

This research was supported in part by a Clinical Research grant from the Virginia-Maryland Regional College of Veterinary Medicine to D.S.L. We thank Cheryl Davis for providing the stock culture of *T. cruzi* and John Dame for the bovine monocyte cell culture.

LITERATURE CITED

BARR, S. C., O. VAN BEEK, M. S. CARLISLE-NOWAK, J. W. LOPEZ, L. V. KIRCHHOFF, N. ALLISON, A. M. ZAJAC, A. DE LAHUNTA, D. H. SCHLAFER, AND W. T. CRANDALL. 1995. *Trypanosoma cruzi* infection in Walker hounds from Virginia. American Journal of Veterinary Research **56:** 1037–1044.

DIAMOND, L. S., AND R. RUBIN. 1958. Experimental infection of certain farm mammals with a North American strain of *Trypanosoma cruzi* from the raccoon. Experimental Parasitology **7:** 383–390.

HERWALDT, B. L., M. J. GRIJALVA, A. L. NEWSOME, C. R. MCGHEE, M. R. POWELL, D. G. NEMEC, F. J. STEURER, AND M. L. EBERHARD. 2000. Use of polymerase chain reaction to diagnose the fifth reported U.S. case of autochthonous transmission of *Trypanosoma cruzi*, in Tennessee, 1998. The Journal of Infectious Diseases **181:** 395–399.

JOHN, D. T., AND K. L. HOPPE. 1986. *Trypanosoma cruzi* from wild raccoons in Oklahoma. American Journal of Veterinary Research **47:** 1056–1059.

KARSTEN, V., C. DAVIS, AND R. KUHN. 1992. *Trypanosoma cruzi* in wild raccoons and opossums in North Carolina. Journal of Parasitology **78:** 547–549.

MCKEEVER, S., G. W. GORMAN, AND L. NORMAN. 1958. Occurrence of a *Trypanosoma cruzi*–like organism in some mammals from southwestern Georgia and northwestern Florida. Journal of Parasitology **44:** 583–587.

PIETRZAK, S. M., AND O. J. PUNG. 1998. Trypanosomiasis in raccoons from Georgia. Journal of Wildlife Diseases **34:** 132–136.

PUNG, O. J., C. W. BANKS, D. N. JONES, AND M. W. KRISSINGER. 1995. *Trypanosoma cruzi* in wild raccoons, opossums, and triatomine bugs in southeast Georgia, U.S.A. Journal of Parasitology **81:** 324–326.

SCHAFFER, G. D., W. L. HANSON, W. R. DAVIDSON, AND V. F. NETTLES. 1978. Hematotropic parasites of translocated raccoons in the southeast. Journal of the American Veterinary Medical Association **173:** 1148–1151.

TELFORD, S. R., JR., AND D. J. FORRESTER. 1991. Hemoparasites of raccoons (*Procyon lotor*) in Florida. Journal of Wildlife Diseases **27:** 486–490.

WALTON, M. J., P. M. BAUMAN, L. S. DIAMOND, AND C. M. HERMAN. 1958. The isolation and identification of *Trypanosoma cruzi* from raccoons in Maryland. American Journal of Tropical Medicine and Hygiene **7:** 603–610.

YABSLEY, M. J., AND G. P. NOBLET. 2002. Seroprevalence of *Trypanosoma cruzi* in raccoons from South Carolina and Georgia. Journal of Wildlife Diseases **38:** 75–83.

———, ———, AND O. J. PUNG. 2001. Comparison of serological methods and blood culture for detection of *Trypanosoma cruzi* infection in raccoons (*Procyon lotor*). Journal of Parasitology **87:** 1155–1159.

J. Parasitol., 91(2), 2005, pp. 472–474

Vertebrate Host Specificity of Two Avian Malaria Parasites of the Subgenus *Novyella*: *Plasmodium nucleophilum* and *Plasmodium vaughani*

Tatjana A. Iezhova, Gediminas Valkiūnas, and Franz Bairlein*, Institute of Ecology, Vilnius University, Akademijos 2, LT-2600 Vilnius, Lithuania; *Institute of Avian Research 'Vogelwarte Helgoland,' An der Vogelwarte 21, 26386 Wilhelmshaven, Germany. *e-mail: tatjana@ekoi.lt*

ABSTRACT: The susceptibility of wild-caught European passeriform birds to naturally isolated malaria parasites, *Plasmodium* (*Novyella*) *nucleophilum* and *Plasmodium* (*Novyella*) *vaughani*, was studied by means of intramuscular subinoculation of infected citrated blood. *Plasmodium nucleophilum* of the great tit, *Parus major*, was transmitted to 3 great tits, but 3 blackcaps (*Sylvia atricapilla*) were not susceptible. *Plasmodium vaughani* of the robin, *Erithacus rubecula*, was transmitted to 1 robin and 1 blackcap, but 1 dunnock, *Prunella modularis*, was not susceptible. The prepatent period was between 8 and 10 days in all experimental infections. Maximum experimental parasitemia (3.4% of red cells) was detected in great tits infected with *P. nucleophilum* 23 days postexposure. A light (<0.01%) transient parasitemia of *P. vaughani* developed in the robin and blackcap. This study is in accord with former experimental observations on host specificity of *P. nucleophilum* and *P. vaughani*, which are characterized by a wide, but selective, range of avian hosts. Two new host–parasite associations were recorded.

Avian malaria parasites (Plasmodiidae, *Plasmodium*) of the subgenus *Novyella* Corradetti, Garnham and Laird, 1963 are some of the most common hematozoa, found particularly in passeriform birds. They are characterized by small meronts (less than the size of the erythrocyte nucleus), scanty cytoplasm, and elongated gametocytes (Corradetti et al., 1963). Ten species in the subgenus have been described, but there is limited information about their life cycles, pathogenicity, and vertebrate host specificity (Telford et al., 1997; Valkiūnas, 1997). Although numerous surveys report *Novyella* species in passeriform birds from all over the world, frequently with a high prevalence of infection (Bishop and Bennett, 1992; Valkiūnas et al., 2003), there are few experimental studies that detail the host specificity of these parasites (Corradetti and Scanga, 1973; Christensen et al., 1983; Telford et al., 1997; Valkiūnas, 1997). In the present paper, we present results that detail the experimental transmission of 2 malaria parasites of the subgenus *Novyella*, i.e., *Plasmodium nucleophilum* Manwell, 1935 and *Plasmodium vaughani* Novy and MacNeal, 1904, which have frequently been reported in wild birds from different families, particularly passeriforms, worldwide (Garnham, 1966; Bishop and Bennett, 1992; Valkiūnas, 1997).

The work was carried out at the Institute of Avian Research 'Vogelwarte Helgoland,' Wilhelmshaven, Germany. In all, 220 passeriform birds belonging to 15 species were caught with mist nets in the environs of the institute in June 2002. Blood was taken by puncturing the brachial vein. Three slides were prepared from each bird. Blood films were air dried, fixed in methanol, and stained with Giemsa. The slides were examined for 10–15 min at low magnification (×400), and then at least 100 fields were studied at high magnification (×1,000). Intensity of infection was estimated as a percentage by actually counting the number of parasites per 1,000 erythrocytes examined and per 10,000 erythrocytes at low parasitemias, i.e., <0.1%, as recommended by Godfrey et al. (1987). Malaria parasites were identified according to Valkiūnas (1997). One great tit, *Parus major*, and 1 robin, *Erithacus rubecula*, that were naturally infected with *P. nucleophilum* and *P. vaughani*, respectively, and 9 uninfected birds of 4 species were held in cages during this study. The infected birds with active parasitemia were used as donors of malaria parasites to infect the recipient birds, which were 3 great tits, 1 robin, 4 blackcaps (*Sylvia atricapilla*), and 1 dunnock (*Prunella modularis*). Blood from the recipient birds was taken once per day for 5 days before subinoculation. The blood smears from each recipient bird were examined for hematozoa at high magnification for 3 hr. At least 1,000,000 red blood cells were examined during this time. Malaria parasites were not seen in them.

All birds were taken under license from the wild and kept indoors under controlled conditions (20 ± 1 C; 50–60% relative humidity; a constant light/dark period of 12/12 hr), given a standard diet (Bairlein, 1986), and protected from vectors.

Uninfected birds were exposed to the parasites by subinoculation of 50 μl of a freshly prepared mixture of infected blood in a 3.7% solution of sodium citrate (1 part of the solution to 4 parts of the blood) into their pectoral muscle as described by Valkiūnas (1997). Parasitemia of *P. nucleophilum* and *P. vaughani* was 6.8 and 1.9%, respectively, in the donor birds on the day of the exposure. The blood was taken as described above once per 2 days during the first 10 days postinfection (PI) and then once per wk for 53 days. The blood films were examined as described above for nonrecipient birds.

Samples of blood from naturally and experimentally infected birds were cryopreserved according to Garnham (1966) for future experimental work. Representative preparations of the blood stages of *P. nucleophilum* (accession numbers 8007–8020, NS) and *P. vaughani* (8021–8029, NS) were stored at the Institute of Ecology, Vilnius University, Lithuania.

It may be asked whether it is justifiable to make conclusions about experimental evaluation of a host range on the basis of the microscopic examination of blood smears from a small number of wild-caught recipient birds. Microscopic examination of blood films is of low sensitivity for diagnosing malaria infections (Forrester et al., 1974). Evidence must be provided that the recipient birds did not have a chronic or subpatent natural infection. Lines of evidence that support our reasoning include the low prevalence of infection in the wild population from which the birds were removed and the examination of multiple smears from multiple dates. Overall prevalence of malaria parasites of the subgenus *Novyella* was 2.3% at the study site, so the probability that the recipient birds had natural malaria infections was low. Extended microscopy (between 2 and 3 hr) has been shown to be effective in detecting low-intensity chronic malaria infections (Demina and Pavlova, 1962; Valkiūnas, 1997). Because intensive microscopy of blood smears was carried out on each of the 5 days before subinoculation, it seems likely that the recipient birds were not infected before their experimental exposure.

Plasmodium nucleophilum of the great tit was transmitted to 3 great tits. Three blackcaps were not susceptible. The prepatent period was 10 days. The maximum parasitemia was detected 23 days PI, when up to 3.4% of erythrocytes were parasitized. Light, but rather constant para-