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# SEROLOGICAL DIAGNOSIS OF *BAYLISASCARIS PROCYONIS* IN PRIMATES USING A HUMAN ELISA TEST

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*Abstract:* The usefulness of a human enzyme-linked immunosorbent assay (ELISA) for serological diagnosis of *Baylisascaris procyonis* larva migrans was assessed in nonhuman primates (NHP). The test was originally developed as an assay performed on human samples at Purdue University. Six participating zoos submitted 258 NHP serum samples, spanning these major phylogenetic groups: 1) great apes ($n = 84$), 2) lesser apes ($n = 17$), 3) Old World monkeys ($n = 84$), 4) New World monkeys ($n = 20$), and 5) prosimians ($n = 53$). Sera were tested in duplicate using a microtiter-well ELISA with *B. procyonis* larval excretory-secretory proteins as antigen, and serum from an experimentally infected baboon (*Papio anubis*) served as positive control. The ELISA clearly identified seropositive animals in all zoos. With putative cutoffs of optical density (OD) measured at 405 nm ($OD_{405}$) of $<0.150$ = negative, 0.150–0.250 = indeterminate, and $>0.250$ = positive, 149 of 258 (57.8%) were clearly negative (mean OD 0.046), and 78 of 258 (30.2%) were clearly positive (mean OD 0.657, range 0.253–1.773), the rest being indeterminate. Of these, 15 were high positive with OD 1.095–1.773 (mean 1.314). Positive animals were seen from all zoos; 76 (97.4%) were great apes, lesser apes, or Old World monkeys. The four highest ODs were in a siamang (*Symphalangus syndactylus*), lion-tailed macaque (*Macaca silenus*), Sumatran orangutan (*Pongo abelii*), and western lowland gorilla (*Gorilla gorilla gorilla*), all from different zoos. Prosimians had a mean OD of 0.039 and New World monkeys 0.021, indicating that human reagents either did not work for these groups or few infected animals were represented. These results indicate that the human ELISA for *B. procyonis* works well for at least higher phylogeny NHP and that serologic evidence of infection is surprisingly common, correlating with what is known for exposure to this parasite in zoos.

*Key words:* *Baylisascaris procyonis*, neural larva migrans, nonhuman primates, serology, zoological institutions.

## INTRODUCTION

*Baylisascaris procyonis*, the raccoon ascarid, is a common intestinal parasite of raccoons that has emerged as an increasingly well recognized helminthic disease of both animals and humans, in which it causes various forms of larva migrans or baylisascariasis. *Baylisascaris procyonis* has a widespread geographic range, with cases more prevalent in North America, Europe, and parts of Asia.[20,21] The risk of infection is thought to be much greater than currently recognized, necessitating the need for a targeted diagnostic methodology.

North American raccoons (*Procyon lotor*) are commonly infected with *B. procyonis*, with prevalence rates up to 100% in some areas.[20,21] Infected raccoons can shed millions of *Baylisascaris* eggs daily, which are extremely resistant in the environment, often remaining infective in soil for years.[20] Raccoons utilize preferred communal defecation sites termed latrines,[25] which occur as focal areas throughout their home range—not uncommonly, including in and around zoological institutions. In zoos, raccoon latrines are typically found in stored hay and straw used to feed or bed animals, in stored grain or other food items, on walkways or sidewalks near enclosures, or within enclosures themselves.[20,21] They are also found frequently on rooftops of buildings and on tops of other enclosures, where the feces and infective eggs can fall into the exhibits below.[20,21,25] Depending on the level of egg ingestion via the fecal-oral route, infection results in a wide spectrum of clinical disease in other animals (paratenic hosts),[21] affecting individuals or larger numbers of animals in outbreaks.[20,21]

Clinically, baylisascariasis is characterized by aggressive larval migration and invasion of the central nervous system (CNS), causing a form of the disease termed neural larva migrans (NLM) or *Baylisascaris* encephalitis.[16,21] The parasite has produced fatal or severe NLM in more than 150 species of paratenic host animals, both birds and mammals, in North America[21] and is a well-recognized zoonosis.[14,16,20,21,24] In many zoos, *Baylisascaris* NLM is an increasingly recognized

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problem, particularly in nonhuman primates (NHPs).[21,31]

However, *Baylisascaris* larva migrans is difficult to diagnose clinically. Because *B. procyonis* does not complete its life cycle in paratenic hosts, eggs and larvae are not shed in their feces, negating diagnosis by fecal examination.[21] In these animals, depending on dose, hematologic and biochemistry values are often within normal limits, although affected animals may have a cerebrospinal fluid (CSF) eosinophilic pleocytosis. Finding larvae in brain biopsies or through advanced imaging (computed tomography, magnetic resonance imaging [MRI]) is usually unrewarding and often impractical in NHPs, although MRI lesions in deep white matter are suggestive.[1,16,17,20,21] In addition to clinical signs and epidemiologic associations, antemortem diagnosis is strongly dependent on finding anti–*B. procyonis* antibodies in serum and CSF, at least in humans.[14,16,21] Polymerase chain reaction (PCR) assays have been developed,[9,13] and one is commercially available (Zoologix Inc., Chatsworth, CA 91311, USA), but are only useful on tissues, feces, or soil that contain larvae or eggs. Larvae are rarely present in the blood in cases of NLM, so PCR may only be practical to confirm infection postmortem or in biopsies if larvae are found in brain or other tissues.

The Department of Comparative Pathobiology at Purdue University developed a functional enzyme-linked immunosorbent assay (ELISA) for *Baylisascaris* larva migrans in humans, which was routinely used as a screening test and in clinical cases.[16] The antigen used in the ELISA was *B. procyonis* excretory and secretory products produced by in vitro–cultured stage 3 (L3) larvae.[3,4,7,30] The ELISA proved to be of great value in aiding the diagnosis of clinical cases of human baylisascariasis and was later combined with western blotting.[7,16] It has been applied to most human cases and hundreds of human samples, including two large groups of children in California and Chicago.[16] Recently, a recombinant antigen-based ELISA and western blotting were developed with a cloned *B. procyonis* larval protein,[8,10,26] but these were unavailable at the time of this study.

This study was designed to determine the seroprevalence of *B. procyonis* in zoo primates, as well as shed light on whether human reagents and cutoff values for the serologic test in humans could also apply to NHPs and, as such, be used as a diagnostic tool for them, as well. Primates were chosen as the focus of this project because of their susceptibility to *Baylisascaris* infection, their high profile and megacharismatic nature in zoo collections, their representation as an evolutionary lineage from lower to higher primates, the availability of human immunodiagnostic reagents for serologic testing, and their often endangered status.

## MATERIALS AND METHODS

Sera were obtained from potentially exposed (or infected) individuals either 1) when taken for routine diagnostic purposes and stored temporarily or historically in serum banks (within the last 20 yr), or 2) prospectively from animals showing clinical signs suggestive of *Baylisascaris* NLM. An anamnesis of the individual and institution was collected for each sample retrospectively, including any reports of clinical signs compatible with *Baylisascaris* NLM (defined as neurological dysfunction of undiagnosed cause). Samples were shipped on ice to Purdue University for testing.

Serological testing for antibodies against *Baylisascaris* was performed using an enzyme-linked immunosorbent assay (ELISA). The ELISA test was an alkaline phosphatase–based microtiter well assay using excretory-secretory antigens derived from in vitro–cultured *B. procyonis* L3 larvae.[3,4,10,11,30] Serum from an experimentally infected baboon (*Papio anubis*) was used as a positive control (optical density [OD] 1.10–1.30). All serum samples were run in duplicate and the mean, standard deviation, and coefficient of variation were calculated.[6,10] The OD of reactions was measured at 405 nm ($OD_{405}$) using a ThermoMax absorbance microplate reader (Molecular Devices, Sunnyvale, CA 94089, USA). Cutoff values were set for a 99% confidence interval (CI) at the mean + 3 SD, as previously described,[6,10] and were calculated using means and standard deviations for negative to low OD groupings in increments up to OD < 0.250 as a "negative" population.[10] This method is routinely used to establish cutoff values in endemic populations where no true negative or true positive samples are known or available.[6] With a standard conservative bracketing (±0.050 OD) of the calculated cutoff as indicating an indeterminate reactor group,[6] putative cutoffs were set as follows at $OD_{405}$: <0.150 = negative, 0.150–0.250 = indeterminate, and >0.250 = positive.

Data on ELISA reactivity (mean $OD_{405}$ values with standard deviations) were compiled and entered into an electronic database. Differences between value groupings within and between NHP species were assessed for significance by

analysis of variance methods (SPSS 22.0; IBM Inc., Armonk, NY 10504, USA) with the known limitation of sample size for some species. Ninety-five percent binomial CIs were utilized. Chi square tests were employed to analyze differences by species, sex, age, diet, and the presence of suspected or confirmed *B. procyonis* NLM. For example, diet was examined to determine whether differences in preferred diet related to seropositivity. The species were placed in four general diet categories based on primary diet composition: primary insectivore (bushbaby [*Otolemur garnettii*], tamarin [*Saguinus midas, S. oedipus*], squirrel monkey [*Saimiri sciureus*]), primary folivore (western lowland gorilla [*Gorilla gorilla gorilla*], colobus [*Colobus guereza, C. angolensis angolensis*], langur [*Trachypithecus francoisi*] , howler monkey [*Alouatta caraya*]), primary frugivore (Sumatran orangutan [*Pongo abelii*], Bornean orangutan [*Pongo pygmaeus*], siamang [*Symphalangus syndactylus*], gibbon [*Hylobates lar, Nomascus leucogenys*], spider monkey [*Ateles geoffroyi*], lion-tailed macaque [*Macaca silenus*], lemur [*Eulemur macaco, Lemur catta, Varecia rubra, Varecia variegata*]), and omnivores (baboon [*Papio hamadryas*], De Brazza's monkey [*Cercopithecus neglectus*], Diana monkey [*Cercopithecus diana*]). Logistic regression was subsequently performed on all variables simultaneously.

## RESULTS

Six cooperating zoos submitted 258 NHP serum samples representing 23 species and spanning five major phylogenetic groups (Table 1): 1) great apes ($n = 84$), 2) lesser apes ($n = 17$), 3) Old World monkeys ($n = 84$), 4) New World monkeys ($n = 20$), and 5) prosimians ($n = 53$). According to the putative OD cutoffs described above, 149 of 258 animals (57.8%) were clearly negative (mean OD 0.046), and 78 of 258 (30.2%) were clearly positive (mean OD 0.657, range 0.253–1.773), the rest being indeterminate. Of positive animals, 15 were high positive, with OD 1.095–1.773 (mean 1.314). Another 21 ranged from 0.530 to 0.998. Positive animals were seen from all zoos, and 76 (97.4%) were great apes, lesser apes, or Old World monkeys (Table 1). The four highest ODs were in a siamang (OD 1.773), lion-tailed macaque (OD 1.642), Sumatran orangutan (OD 1.628), and western lowland gorilla (OD 1.429), all from different zoos. Prosimians had a mean OD of 0.039 and New World monkeys of 0.021, with lower seroprevalences of 1.9% and 5%, respectively ($P < 0.001$).

Of the primates that were 30+ years old, 57.1% had OD > 0.250, which was higher than any other age group, ranging from 21.8% to 33.3% positive. One gorilla was of unknown age at the time of sampling.

Seroprevalence between genders was not significantly different. Of the females in this study, 41 of 140 (29.3%) were positive, whereas 37 of 118 (31.4%) males were positive.

When diet was examined as a potential influential factor of seropositivity, 44.2% of the folivores were positive, which was higher than any other group (Fig. 1). The other categories ranged from 16.7% to 26.3% positive.

Few clinical cases of suspected or confirmed NLM were included in this study, but it was interesting to note that the animal with the highest OD (siamang, 1.773) was not clinically affected, at least not at the time of sampling. Animals with clinical signs consistent with baylisascariasis were much more likely to be seropositive (5 of 6, 83.3%), with ODs ranging from 0.005 to 1.581, whereas animals with no clinical signs were more likely to be seronegative (148 of 252, 58.7%), with ODs ranging from 0.001 to 1.773 ($P = 0.003$).

## DISCUSSION

NHPs are highly susceptible to *Baylisascaris* larva migrans and, to date, at least 31 NHP species have been identified with proven or suspected *Baylisascaris* NLM by postmortem examination (most cases), biopsy, positive serology, or a combination of methods, including results from this study.[1,2,5,12,15,17–23,28,29,31] Great apes, lesser apes, and Old World monkeys were more likely to be positive, at 44.0%, 52.9%, and 35.7%, respectively, by human ELISA for *B. procyonis.*

Prosimians and New World monkeys had substantially lower mean ODs, and further investigation of these groups is warranted. The results could indicate that human reagents either did not work for these "lower" primate groups or that too few infected animals were represented, or they could be influenced by age, as these species have shorter life spans than other NHPs and, thus, less time to be exposed. Other factors likely include behavioral and dietary differences between primate groups, which might influence their direct exposure to raccoon latrines or contaminated materials and ingestion of infective eggs.

Most human cases of *Baylisascaris* NLM with severe clinical signs had positive ODs in the 1.30–3.20 range;[16] values at the high end of this range were not seen in this study. However, clinically affected primates confirmed on histopathology

**Table 1.** Distribution of serum optical density measured at 405 nm ($OD_{405}$) of nonhuman primates categorized by species within phylogenetic groups by human ELISA for *Baylisascaris procyonis* larva migrans.

| Phylogenetic group | Positive OD >0.250 | Indeterminate OD 0.150–0.250 | Negative OD <0.150 | Total | % positive |
|---|---|---|---|---|---|
| Great ape | 37 | 18 | 29 | 84 | 44.0 |
| *Gorilla gorilla gorilla* | 28 | 13 | 8 | | |
| *Pongo abelii* | 9 | 5 | 20 | | |
| *Pongo pygmaeus* | 0 | 0 | 1 | | |
| Lesser ape | 9 | 5 | 3 | 17 | 52.9 |
| *Hylobates lar* | 1 | 3 | 1 | | |
| *Nomascus leucogenys* | 4 | 2 | 2 | | |
| *Symphalangus syndactylus* | 4 | 0 | 0 | | |
| Old World monkey | 30 | 6 | 48 | 84 | 35.7 |
| *Cercopithecus diana* | 6 | 1 | 1 | | |
| *Cercopithecus neglectus* | 2 | 0 | 0 | | |
| *Colobus angolensis angolensis* | 0 | 0 | 3 | | |
| *Colobus guereza* | 9 | 0 | 2 | | |
| *Macaca silenus* | 10 | 2 | 7 | | |
| *Papio hamadryas* | 2 | 3 | 22 | | |
| *Trachypithecus francoisi* | 1 | 0 | 13 | | |
| New World monkey | 1 | 0 | 19 | 20 | 5.0 |
| *Alouatta caraya* | 0 | 0 | 10 | | |
| *Ateles geoffroyi* | 0 | 0 | 5 | | |
| *Saguinus midas* | 1 | 0 | 0 | | |
| *Saguinus oedipus* | 0 | 0 | 3 | | |
| *Saimiri sciureus* | 0 | 0 | 1 | | |
| Prosimian | 1 | 2 | 50 | 53 | 1.9 |
| *Eulemur macaco* | 0 | 1 | 26 | | |
| *Lemur catta* | 0 | 1 | 1 | | |
| *Otolemur garnettii* | 0 | 0 | 1 | | |
| *Varecia rubra* | 0 | 0 | 2 | | |
| *Varecia variegata* | 1 | 0 | 20 | | |
| Total | 78 | 31 | 149 | 258 | 30.2 |

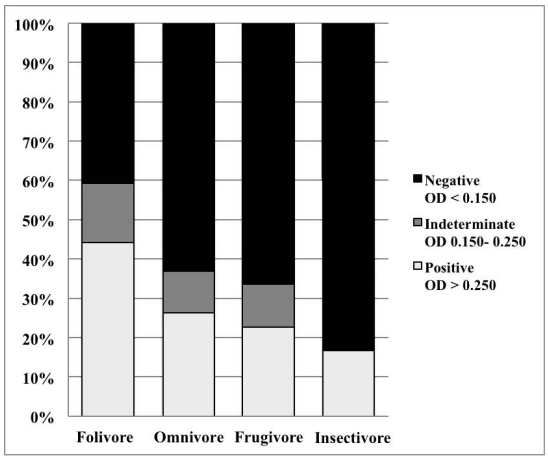


**Figure 1.** Serum optical densities measured at 405 nm ($OD_{405}$) of nonhuman primates, categorized by dietary preference by human ELISA for *Baylisascaris procyonis* larva migrans.

exhibited high-positive ODs within this range, and the positive control, an experimentally infected baboon that developed fatal NLM, had an OD range of 1.10–1.30. Using these NHPs as sentinels, the seroprevalence found in this study further indicates the widespread nature of exposure to *B. procyonis* for other animals and people. Exposure to *Baylisascaris* in zoological institutions appears to be a significant problem, and preventive measures should be implemented whenever possible.[20,21]

In this study, primates over 30 years of age tended to exhibit higher ODs ($P = 0.07$), which correlated with longer living ape species with a tendency for greater reactivity. However, this warrants further study with data-driven age groupings and larger samples sizes. It is noteworthy that in human studies, prevalence of positive reactivity increases with age, presumably related to increased exposure over time.[16]

In the present study, the seroprevalence of *Baylisascaris* in the select NHPs tested varied

from 3 of 22 (14%) to 26 of 47 (55%) across the six zoos, indicating significant exposure in all of these facilities. Variations seen between zoos would be related to local raccoon numbers and *B. procyonis* prevalence, as well as particular facility designs and management, including such things as amount of vegetation and trees in and around exhibits, fencing and other exclusionary practices, food and bedding storage, and so on. Those practices that would encourage or foster raccoon presence on zoo properties would in turn result in increased localized contamination and exposure of resident animals.[20,21,31] NHP behavior should also be considered, in that strictly arboreal primates might be less likely to encounter raccoon feces. Some of the variation seen between zoos could also be due to confounding factors, such as having fewer apes and Old World monkeys (or more prosimians and New World monkeys) at certain institutions. Almost certainly, increased seroprevalence is related to exhibit design and husbandry, in relation to NHP access to raccoon latrines or contaminated materials. For example, all seven lion-tailed macaques from one zoo had high ODs (0.853–1.642, mean 1.279) indicating a probable common source of infection in this group. At another zoo, latrines were discovered on the ledge of an exhibit housing colobus monkeys, of which 9 of 11 (81.8%) were seropositive. Mitigation of this risk would include raccoon control and cleaning up latrines at least weekly, before the eggs become infective (as early as 11–14 days after shedding).[20,21]

Diet was also thought to be a factor that could potentially influence *Baylisascaris* seroprevalence, as folivorous or frugivorous animals may be more likely to forage on vegetation, including grass and leaves in their exhibits, that may be contaminated with raccoon feces or ingest feces that may contain undigested seeds and berries. However, no significant difference was observed between frugivores and folivores, insectivores, or omnivores, perhaps related to sample size.

Animals with clinical signs were more likely to be seropositive ($P = 0.003$), with ODs ranging from 0.005 to 1.581; however, the individual with the highest OD (1.773) and many other seropositive NHPs were asymptomatic (OD range 0.001–1.773). Seropositive animals with no clinical signs are medically termed "covert" infections (i.e., covert *Baylisascaris* or baylisascariasis, similar to covert toxocariasis in humans).[32] Approximately 14% of the human population of the United States have antibodies to *Toxocara* but do not exhibit clinical signs,[33] and seropositive covert baylisascariasis has been found numerous times.[14,16,20,21,27] Asymptomatic infection is the most common form,[16,21] and clinical presentation is determined by the number of eggs ingested, the number of larvae entering the CNS, the site and extent of larval migration, and the size of the host brain.[16,20,21] A common misconception concerning *Baylisascaris* is that the larvae are neurotropic and always cause clinical NLM. They are not neurotropic (i.e., they do not have a predilection for the brain), but some (estimated at 5%–7%) enter the CNS as a result of dissemination.[16,20,21] The other 93%–95% of larvae migrate and encapsulate in various noncritical tissues, where they cause little to no recognizable harm or clinical signs but are still present to stimulate positive serologic responses. Therefore, finding asymptomatic animals with anti-*Baylisascaris* antibodies is not unusual and reflects being exposed to smaller inocula of eggs without CNS migration, having fewer numbers of larvae entering larger brains, where they may be better tolerated, or both conditions. This confers that, similar to humans, low levels of infection occur more commonly in NHPs and other animals than is recognized[21] and that most infections will not result in clinical signs.

Therefore, a positive or negative OD has to be interpreted with caution in light of clinical findings and history. Additionally, the ELISA used in this study detected immunoglobulin G antibodies, indicating only that the animal was infected at some point in time. Antibodies can persist for a long time and, depending on the degree of infection and reinfection, may be present for many months; however, the titer may also decrease over time without further stimulation or rebound because of an anamnestic response after reinfection. Antibody response to *B. procyonis* is related to larval infection and antigenic stimulation and might not correlate directly with clinical signs, which can vary from very subtle signs with slow onset to marked, rapidly progressive disease, depending on levels of infection and CNS migration.[14,16,20,21,24]

Because of the increasing recognition of clinical *B. procyonis* in zoo species, the need for targeted diagnostic methodology has become apparent. The results of this study indicate that serologic evidence of *Baylisascaris* infection is surprisingly common in NHPs in zoos, which correlates with reported cases from zoos and what is known concerning exposure to this parasite in these environments. The human ELISA for *B. procyonis* works well in at least higher phylogeny NHP, but

must be interpreted in light of the aforementioned caveats. It is very important for zoos to be on guard concerning this parasite and to take appropriate measures to protect NHPs and other resident animals from infection.

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