A recombinant antigen-based enzyme-linked immunosorbent assay for specific diagnosis of
Baylisascaris procyonis larva migrans

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Running title: B. procyonis RAG1 ELISA

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Baylisascaris larva migrans is an important zoonotic disease caused by Baylisascaris procyonis, the raccoon roundworm, and is being increasingly considered in the differential diagnosis of eosinophilic meningoencephalitis in children and young adults. Although B. procyonis excretory-secretory (BPES) antigen-based ELISA and Western blot assays are useful in the immunodiagnosis of this infection, cross-reactivity remains a major problem. Recently, a recombinant B. procyonis antigen, BpRAG1, was reported for use in development of improved serological assays for the diagnosis of Baylisascaris larva migrans. In this study, we tested a total of 384 human patient serum samples in a BpRAG1 ELISA, including 20 patients with clinical Baylisascaris larva migrans, 137 patients with other parasitic infections (8 helminth and 4 protozoan), and 227 with unknown/suspected parasitic infections. A sensitivity of 85% and specificity of 86.9% was observed with the BpRAG1 ELISA, compared to only 39.4% specificity with the BPES ELISA. In addition, the BpRAG1 ELISA had a low degree of cross-reactivity with antibodies to Toxocara spp. infection (25%), while the BPES antigen showed 90.6% cross-reactivity. Based on these results, BpRAG1 antigen has a high degree of sensitivity and specificity and should be very useful and reliable in the diagnosis and seroepidemiology of Baylisascaris larva migrans by ELISA.

INTRODUCTION

Baylisascaris larva migrans is an important zoonotic disease caused by the raccoon roundworm, Baylisascaris procyonis (17, 22). The disease manifests as visceral (VLM), ocular (OLM), and/or neural larva migrans (NLM), related to tissue damage and inflammation caused by aggressive migration of B. procyonis larvae within the human host (1, 14, 16). Similar to other geohelminth zoonoses, the infection occurs following accidental ingestion of infective B. procyonis eggs from
areas contaminated with raccoon feces (14). Raccoons typically defecate in preferred sites called latrines. These latrine sites and their surrounding areas become heavily contaminated with infective *B. procyonis* eggs and pose a significant risk of infection to small mammals, birds, and humans. Raccoon latrines are commonly found on logs, at the base of trees, on large tree limbs or rocks, but may also occur on rooftops, in playgrounds, recreational parks, sandboxes, and other areas (23). Children have a higher risk of infection due to their inquisitive nature, exploration of their surroundings and a tendency to put contaminated materials in their mouth.

Although this infection is known to occur in Europe and parts of Asia, most reported cases are from North America (United States and Canada), where raccoons are both common and widely distributed (10, 22).

Clinical signs and symptoms associated with migrating *B. procyonis* larvae are often non-specific, although there is a greater association with the production of eosinophilic meningoencephalitis (24). Current diagnosis of *Baylisascaris* larva migrans is based on a combination of criteria, including the patient’s history of exposure to raccoons or raccoon feces, clinical signs consistent with larva migrans (particularly NLM) and results of clinical laboratory tests. These include eosinophilia in peripheral blood and cerebrospinal fluid, and positive serology (anti-*B. procyonis* IgG detection, performed at Purdue University) using *B. procyonis* larval excretory-secretory (BPES) antigen-based ELISA and Western blot assays (6). Although larval recovery and identification is the gold standard for diagnosis, there is a low probability of detecting larvae in brain biopsy samples, and fewer of these invasive procedures are being done with the availability of serologic testing (10, 22). In conjunction with other criteria listed above, serologic tests are performed to assist the diagnosis of clinical *Baylisascaris* larva migrans, especially NLM and OLM. However, covert infections with *B. procyonis*, showing mild or no
symptoms, can also be expected in relatively large numbers based on the widespread distribution of raccoons in North America, the high prevalence of this parasite (68->90%) in raccoon populations, and the level of human exposure to *B. procyonis* eggs (14, 17). Prior to the knowledge of cross-reactivity in the BPES ELISA, an 8% seroprevalence of *B. procyonis* infection was reported in children in the Chicago area (3), and may have been affected somewhat by concomitant *Toxocara* infections in the population.

Studies of serologic diagnosis of *Baylisascaris* larva migrans, using BPES antigen-based ELISA and Western blots, have shown that cross-reactivity occurs with *Toxocara* spp. and other ascariid infections (2, 6). Cross-reactivity is a common hurdle in the development of serodiagnostic tests with higher specificity. Serodiagnostic tests developed for various nematodes (including *Toxocara* spp.), using both crude somatic and excretory/secretory (ES) antigens, have demonstrated high sensitivity but often show lower specificity, related to varied levels of cross-reactivity (8, 9, 11, 13). Western blot assays have some advantage over ELISA in separating cross-reacting versus parasite-specific antigens (11, 20), but are logistically more difficult and time consuming to perform. Currently available serodiagnostic tests for *Baylisascaris* larva migrans include a combination of highly sensitive BPES antigen-based ELISA and Western blot assays, the latter in which *Baylisascaris*-specific 30-45 kDa ES antigens are recognized by serum from *B. procyonis* infected individuals (6). Serodiagnostic tests using recombinant antigens have shown increased specificity in the diagnosis of different parasitic infections, including *Toxocara* larva migrans (21, 26). In addition to possessing high specificity, these recombinant antigens overcome the various limitations involved in the preparation of ES antigens, and obviate the possible infection risk to those involved in generating this material.
Toxocara spp. larva migrans is known to occur commonly in the United States, where the national seroprevalence is currently 14% (25). Toxocariasis is the most important parasitic infection that needs to be serologically differentiated from B. procyonis, because both parasites overlap with a similar epidemiology in temperate regions, and both infections show similar non-specific as well as clinical symptoms. Recently, a recombinant B. procyonis larval excretory-secretory antigen, RAG1 (rRAG1), with considerable diagnostic potential was reported for use in the development of improved serological assays for diagnosis of Baylisascaris larva migrans (7). This BpRAG1 antigen did not cross-react with anti-Toxocara canis or anti-Ascaris suum antibodies raised in rabbits, and showed great potential for use in ELISA testing. Since this BpRAG1 antigen does not cross-react against antibodies to Toxocara spp. infection, it will also overcome the problem of one-way cross-reactivity observed with BPES antigen and should be of great utility in the diagnosis of Baylisascaris larva migrans.

In the present study, we examined the use of this BpRAG1 antigen in a diagnostic ELISA for Baylisascaris larva migrans. We determined the diagnostic sensitivity and specificity of this BpRAG1 ELISA, based on the reactivity of serum samples from patients with Baylisascaris larva migrans, Toxocara larva migrans, and a variety of other parasitic infections. In addition, we report the results of testing 227 serum samples from patients with unknown or suspected parasitic infections.

MATERIALS AND METHODS

Preparation of BPES and BpRAG1 antigens

Collection, preservation and in vitro embryonation of B. procyonis eggs were performed as per Kazacos et al. (18). Second stage larvae (L2) were hatched aseptically from in vitro-
embryonated eggs, and larval cultures established and processed at weekly intervals (2, 6).

Briefly, the culture medium containing the ES antigen of *B. procyonis* larvae was collected and
dialyzed against 0.1M ammonium bicarbonate solution. The dialyzed antigen was concentrated
by lyophilization, aliquoted and stored at -20°C until use.

The BpRAG1 antigen was prepared as per the protocol described previously (7). Briefly,
the polyhistidine-tagged BpRAG1 protein was expressed in BL-21(DE3) pLysS *E.coli* cells and
purified under denaturing conditions. The eluted protein fractions were extensively dialyzed
against phosphate buffered saline at 4°C, aliquoted and stored at -80°C.

**Serum samples**

(i) **Positive and negative control sera**

Positive control serum was obtained from the Division of Parasitic Diseases, CDC,
Atlanta GA, and consisted of serum from an experimentally infected baboon that developed
severe NLM following infection with *B. procyonis* embryonated eggs (6). Negative control
serum was from a healthy adult human with no history of exposure to raccoons or any clinical
signs of infection.

(ii) **Human serum samples**

a) **Sera from patients with clinical *Baylisascaris* larva migrans**

Serum samples from 20 individuals who were diagnosed with clinical *Baylisascaris* larva
migrants and were determined to be seropositive by BPES Western blot assay (6) were used as
*Baylisascaris*-specific human sera to evaluate the sensitivity of the BpRAG1 ELISA. The criteria
upon which these sera were considered as *Baylisascaris*-specific have been described previously
(6).

b) **Sera from patients with other parasitic infections**
115 serum samples from patients with 12 different parasitic infections, viz, *Toxocara*, *Strongyloides*, *Trichinella*, filariasis, *Schistosoma*, *Fasciola*, *Taenia*, *Echinococcus*, *Trypanosoma*, *Entamoeba*, *Leishmania*, and *Plasmodium* were obtained from the National Reference Centre for Parasitology, McGill University Health Centre, Montreal, Quebec and used to assess the specificity of both the BpRAG1 and BPES ELISAs. Cross-reactivity of BPES antigen to different parasitic diseases (except *Toxocara* spp.) is not known and therefore was evaluated during this study. In addition to these 115 samples, 22 serum samples from patients positive for *Toxocara* larva migrans (identified by testing in the *Toxocara* EIA), previously obtained from the CDC and tested in the BPES Western blot assay (6), were also used in this study.

c) **Unknown/suspected parasite serum samples submitted for serology**

227 serum samples from human patients (either sex and different age groups) primarily from the United States and Canada were submitted to the Parasitology Laboratory, Purdue University, West Lafayette, IN during the period 1986 to 2008 to test for *Baylisascaris procyonis* infection/antibodies using BPES antigen-based ELISA or immunofluorescence. These patients had a history of exposure to raccoons or raccoon feces and/or symptoms possibly associated with larva migrans or a clinical laboratory test indicating blood or CSF eosinophilia. Neither exposure history nor clinical symptoms necessarily meant they had *Baylisascaris* infection. Hence, all samples were treated as coming from patients with unknown or suspected parasite infection. These samples were tested in the BpRAG1 ELISA and compared to the BPES ELISA results.

**Enzyme-linked immunosorbent assay and Western blot**
Checkerboard titrations were done to determine optimum well-coating amounts of antigen, blocking agent, and dilutions of primary and secondary antibodies in the ELISAs (5). *Baylisascaris procyonis* ES antigen and BpRAG1 antigen at concentrations of 0.1 µg and 0.125 µg per well, respectively, were used to coat wells of Immulon2HB flat-bottom microtiter plates (Thermo Scientific, Asheville, North Carolina), and ELISA was performed as described previously (7) with a few modifications. Primary antibody (human patient sera) was used at 1:200 dilution in the BPES ELISA and at 1:100 dilution in the BpRAG1 ELISA, respectively. Alkaline phosphatase-conjugated goat anti-human IgG (H+L) (Bethyl Laboratories, Inc., Montgomery, Texas) was used as the secondary antibody and para-nitrophenyl phosphate (Sigma-Aldrich, St. Louis, Missouri) as the substrate. Microtiter plates were read in a THERMOmax absorbance microplate reader (Molecular Devices, Sunnyvale, California) at 405 nm. All sera were run in duplicate and results averaged. Western blot assays using BPES antigen were performed on representative patient sera that tested positive in the BPES ELISA, according to the protocol described previously (6), to further determine the specificity of BPES antigen for diagnosis of *Baylisascaris* larva migrans as well as to identify cross-reacting ES components.

**Determination of cutoff values and parameters**

Cutoff values for the BPES ELISA were based on an analysis of multiple sets of sera obtained from children and adults, including (1.) 251 random sera collected from 5-7-year old children in Chicago in 2001; (2.) 84 sera primarily from children in southern California in 2002, associated with a case of NLM; and (3.) 201 sera from all age groups submitted to our laboratory in the 1990s-early 2000s for testing purposes, which included some of the 227 sera mentioned above. Using calculated means and standard deviations (SD) for negative to low OD groupings
(in increments up to OD <0.250) as “negative” populations, and the customary practice of setting
cutoffs for a 99% confidence interval at the Mean+3SDs (5), a cutoff value of approximately OD
0.200 was determined for all three sera groups, respectively. Since a true cutoff is never known,
a prudent and conservative practice involves bracketing the calculated cutoff as a suspect or
indeterminate reactor group (5), which was done with +/- OD 0.050. The following parameters
were thus set for the BPES ELISA for use in this study, and until such time as it is in more
routine use, better negative population sera is obtained, and/or other analyses such as Receiver
Operating Characteristic (ROC) curves or J-index analysis (27) can be done: Serum samples with
OD <0.100 were considered negative, those 0.100-0.150 probable negative, those >0.150-0.250
suspect reactors, and those >0.250 were considered positive. Similar determinations were done
for the rRAG1 ELISA, using 207 sera submitted to our laboratory, which included the 201 sera
of group 3 above. Based on its greater sensitivity and specificity, average mean+3SD values
were lower for this ELISA, and a cutoff value of approx. OD 0.175 determined for incremental
OD groupings <0.250. Taking this into account, and setting a slightly narrower suspect reactor
bracket (+/- OD 0.025) (5), the following parameters were set for the rRAG1 ELISA: Samples
with OD <0.100 were considered negative, those 0.100-0.150 probable negative, those >0.150-
0.200 suspect reactors, and those >0.200 were considered positive.

RESULTS

Diagnostic sensitivity of BpRAG1 ELISA

All 20 B. procyonis-specific sera reacted with moderate to high OD values in the BPES
ELISA, with an average OD of 1.811 (range 0.326 to 3.132) and 16 samples >1.000. When these
20 sera were tested in the BpRAG1 ELISA, 17 samples reacted positive or suspect resulting in
85% sensitivity. One of the 20 samples was determined negative in the BpRAG1 ELISA and two
samples were considered probable negatives. Of the 17 samples that reacted strongly, a majority of 6 samples had ODs between 0.500 and 1.000, while 5 samples had ODs above 1.000 (Table 1, Fig1).

**Diagnostic specificity comparison of BpRAG1 and BPES ELISAs**

A high diagnostic specificity of 86.9% was obtained for the BpRAG1 ELISA compared to a 39.4% specificity of the BPES ELISA, when serum samples from individuals positive for different helminth and protozoan diseases were examined in the two ELISAs. Cross-reactions in the BpRAG1 ELISA were mainly observed with other nematode [19.4% (12 of 62 samples)] and trematode [15% (3 of 20 samples)] infections (Table 2). On the other hand, although cross-reactions observed in the BPES ELISA were also mainly due to nematode and trematode infections, the degree of cross-reactivity was much higher, with 77.4% (48 of 62 samples) and 85% (17 of 20 samples) positive reactions with different nematode and trematode infections, respectively (Table 2). The BPES ELISA also showed cross-reactivity (45%) with serum from patients with cestode infections, while no cross-reactivity was observed in the BpRAG1 ELISA. Although only 25.7% cross-reactivity was observed in the BPES ELISA with serum from patients with protozoan infections, the BpRAG1 ELISA was comparatively more specific with only 8.6% cross-reactivity. BPES Western blot assays were done on representative serum samples from patients with different parasitic infections that showed high ODs on the BPES ELISA, and confirmed that their reactions were due to cross-reactivity (data not shown).

**Cross-reactivity with Toxocara spp. infection in BpRAG1 and BPES ELISAs**

Among the different nematode infections tested in the two ELISAs, a high degree of cross-reactivity occurred with *Toxocara* spp. infections. Cross-reactivity in the BPES ELISA with *Toxocara* spp. infections was previously known, and the BpRAG1 ELISA showed much
Of the 32 toxocariasis samples tested in the two ELISAs, the rRAG1 ELISA showed only 25% (8/32 samples) cross-reactivity as opposed to 90.6% (29/32 samples) cross-reactivity for the BPES ELISA, and almost all of the OD values were low or borderline positive (Table 2, Fig 1). A single sample showed a very high OD (2.821) on BpRAG1 ELISA, and since this sample also recognized 30-45 kDa proteins on a BPES Western blot (6), it represented a patient with a dual infection with both parasites.

Reactivity of unknown/suspected serum samples in BpRAG1 and BPES ELISAs

A total of 227 unknown/suspected serum samples were run in the BpRAG1 and BPES ELISAs. Based on the cutoffs that were set for these two ELISAs, a large proportion (89%) of samples were negative in the BpRAG1 ELISA, compared to 59.5% samples being negative in the BPES ELISA. Sixty-three samples with an OD > 0.250 were determined as positive in the BPES ELISA, with a mean OD of 0.693, while only nine samples with an OD > 0.200 were considered positive in the BpRAG1 ELISA, with a mean OD of 0.235. 29 samples were considered suspect reactors in the BPES ELISA, with ODs from 0.150-0.250, whereas 16 samples were considered suspect reactors in the BpRAG1 ELISA, with ODs from 0.150-0.200 (Table 3).

DISCUSSION

Cross-reactivity is a major hurdle in the development of serological tests with high specificity for the diagnosis of parasitic diseases. In this study, we have demonstrated the high sensitivity and very low cross-reactivity of a recombinant *Baylisascaris* antigen, BpRAG1, and its utility in the diagnosis of *Baylisascaris* larva migrans in human patients. The BpRAG1 antigen showed a sensitivity of 85%, with 17 of 20 samples reacting in this ELISA. Obtaining gold-standard parasite-specific human sera is difficult; however, great efforts were made in defining
Baylisascaris-specific human sera used in this study. Although multiple parameters, such as exposure history, clinical symptoms, autopsy or biopsy findings, epidemiology, and positive serology in the BPES ELISA were used to define the samples as true positives, a high positive reaction in the BPES ELISA but not in the BpRAG1 ELISA is attributed to cross-reactivity of BPES antigen with antigens of other co-infecting geohelminths as well as some other parasites. Larval ES antigen is a heterogeneous mixture of glycoproteins released by metabolically active larvae. Protein sharing, epitope sharing, and/or the presence of similar sugar moieties on the proteins are some reasons for the cross-reactivity seen with the use of ES antigens (19). Recombinant antigen, on the other hand, is a single protein that is non-glycosylated when produced in E. coli, resulting in less or no cross-reactivity.

The BpRAG1 antigen had minimal reactivity with sera from patients with other parasitic diseases and demonstrated a high degree of specificity (86.9%) compared to the BPES antigen (39.4%) in the respective ELISAs. A similar study evaluating a recombinant Toxocara antigen (26) demonstrated 44.4% specificity using Toxocara ES antigen while use of the recombinant antigen at the same concentration showed almost no cross-reactivity. The BpRAG1 antigen still needs to be evaluated for potential cross-reactivity against Ascaris lumbricoides, anisakid infections and some others; however, since BpRAG1 did not cross-react with anti-Ascaris suum antibodies raised in rabbits (7), we would expect it to show minimal cross-reactivity to these other parasites. One drawback of using recombinant antigens for serodiagnostic assays could be lowered sensitivity when compared to ES antigen because the recombinant antigen is a single protein. However, combinations of recombinant antigens are being successfully used to improve the sensitivity of recombinant antigens for serodiagnosis of parasitic infections (21). High cross-reactivity using BPES antigen might not be of much concern considering the absence or very low
prevalence of other helminth infections in the United States, Canada, Europe, etc. However, however, there is a possibility of background titers to other geohelminth infections being present in immigrant populations and travelers, and in areas of the world where *Ascaris* and/or other geohelminths are prevalent. Therefore, the BpRAG1 ELISA would have greater utility than the BPES antigen ELISA in any geographical region where serodiagnosis of baylisascariosis is sought.

Some of the parasite infection serum samples used for specificity testing in the BpRAG1 ELISA were thought to be cross-reacting with the BpRAG1 antigen, however, we speculate that these could be false positive reactions to co-purified *E.coli* antigens in the purified fraction of BpRAG1 antigen. Similar false positive reactions were observed in our previous study (7) involving sera raised against different ascarid species in experimentally infected rabbits. Adsorbing these sera with *E.coli* antigens prior to their use in ELISA testing eliminated these reactions. Owing to the large number of sera involved, this wasn’t done in this study. In the long term, this issue can be overcome by either using improved purification techniques or perhaps by using RAG1-based peptide antigens in the ELISA. Occasional false positive reactions in the BpRAG1 ELISA were also evident by the fact that the same serum samples were not simultaneously positive in both BpRAG1 and BPES ELISA specificity testing, indicating a discrepancy. In addition, when unknown or suspected serum samples were run in the BpRAG1 ELISA, as expected a fairly large percentage of these samples (89%) were negative compared to the BPES ELISA (59.5%). Similarly, 92 of the unknown/suspected samples tested in the BPES ELISA were positive compared to only 25 samples in the BpRAG1 ELISA. Although there is a low prevalence of other helminth infections in the U.S. population, recently a 14% national seroprevalence of *Toxocara* spp. was documented (25). Considering the facts that the
unknown/suspected samples used in this study were submitted to our laboratory from across the United States, that background *Toxocara* titers are common in the population, and that there is a known cross-reactivity of BPES antigen with *Toxocara* antibodies, then the observed 40.5% positive reactors in the BPES ELISA (Table 3) should be interpreted with caution. The percentage of positive reactors observed in the BpRAG1 antigen ELISA was 11%, which is much more in line with what would be expected, and similar to what is known for *Toxocara* spp. (25), which has a similar level of exposure.

In endemic areas, there is the real possibility of exposure of people to infective eggs of both *Baylisascaris* and *Toxocara* from the same environments, due to the commonality of their respective hosts and their close association with humans (10, 22). In addition, dogs sometimes develop patent *Baylisascaris* infections and could contaminate domestic environments and neighborhoods with the eggs of both parasites (12, 14, 15). People also could be exposed from infected kinkajous, which are related procyonids sometimes kept as exotic pets (4, 14). Luckily, the prevalence of patent *Baylisascaris* in dogs appears to be low and pet kinkajous relatively uncommon, so despite the possibility of occurrence, the main concern will continue to be contamination from peridomestic or pet raccoons. The BpRAG1 ELISA showed high specificity and little cross-reactivity to *Toxocara* spp. infection in humans and therefore can be used in the differential serodiagnosis of larva migrans caused by these two parasites. The BpRAG1 ELISA will be a superior test in cases of larva migrans caused by concurrent infection with these two parasites, as compared to the combination of BPES ELISA, *Toxocara* ELISA and Western blot assay recommended previously (6). In a previous study, a high-titered *Toxocara* ELISA-positive serum sample was suspected of dual infection with *Baylisascaris* and *Toxocara*, based on its recognition of 30-45 kDa BPES antigen components in a Western blot assay (6). Given the high
cross-reactivity observed with the BPES ELISA, it was not unexpected that we obtained a strong
reaction with this same sample (OD 2.249); however, this particular serum sample also showed
strong reactivity in the BpRAG1 ELISA (OD 2.821; outlying sample #1 on Fig 1), confirming
dual infection in this patient and the utility of the BpRAG1 ELISA.

In conclusion, this study clearly showed a high sensitivity and specificity of the BpRAG1
antigen for the serodiagnosis of *Baylisascaris* larva migrans, including low or no cross-reactivity
to other parasites, including *Toxocara* spp. In endemic areas, all patients suspected of larva
migrants should be tested for antibodies against both *Baylisascaris* and *Toxocara* spp. Testing for
anti-*B. procyonis* antibodies using the BpRAG1 antigen ELISA is much easier than performing a
combination of BPES ELISA and Western blotting, and the BpRAG1 antigen has great promise
for use in diagnostic applications and seroepidemiological investigations. Finally, we wish to
inform the scientific community that ELISA testing for *Baylisascaris* has been discontinued by
our laboratory at Purdue University, and that in the public interest such testing including the
BpRAG1 antigen ELISA has been transferred to the U.S. Centers for Disease Control and
Prevention in Atlanta, GA and the Canadian National Reference Centre for Parasitology in
Montreal, QC, both of which will undertake serologic testing for *Baylisascaris* in the near future.

**REFERENCES**

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   larval excretory-secretory antigens of *Baylisascaris procyonis*, *Toxocara canis* and


FIG 1. Reactivity of serum samples from patients with *B. procyonis* and *Toxocara* spp. larva migrans on *B. procyonis* ES antigen and BpRAG1 ELISAs. Serum from patients with *Toxocara* infection showed strong reactivity on the BPES ELISA (0.250 OD cutoff) indicating cross-reactivity, but few of them reacted on the BpRAG1 ELISA indicating low cross-reactivity on that assay (0.200 OD cutoff). The single very strong reactor is considered a dual infection (→) with both parasites, which was also confirmed by Western blotting.
TABLE 1. Sensitivity of BpRAG1 ELISA for diagnosis of *Baylisascaris* larva migrans.

<table>
<thead>
<tr>
<th>Category</th>
<th>Baylisascaris procyonis recombinant RAG1 antigen ELISA</th>
<th>Optical Density at 405nm</th>
<th>No. of samples (n=20)</th>
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<tr>
<td>Negative</td>
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<td>Probable negative</td>
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<tr>
<td>Suspect reactor</td>
<td>&gt;0.150 - 0.200</td>
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<td></td>
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<tr>
<td>Positive</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>0.500-1.000</td>
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<td></td>
<td>1.000-1.500</td>
<td>2</td>
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<tr>
<td></td>
<td>&gt;1.500</td>
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TABLE 2. Specificity comparison of BPES and BpRAG1 ELISAs for serodiagnosis of *Baylisascaris* larva migrans

<table>
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<tr>
<th>Infection group</th>
<th>Number of serum samples tested</th>
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<th>BpRAG1 ELISA results</th>
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<td></td>
<td>No. negative</td>
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<td><strong>14</strong></td>
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<td><strong>Subtotal</strong></td>
<td><strong>20</strong></td>
<td><strong>11</strong></td>
<td><strong>9</strong></td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amebiasis</td>
<td>10</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Leishmaniasis</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Malaria</td>
<td>10</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Chagas</td>
<td>10</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td><strong>35</strong></td>
<td><strong>26</strong></td>
<td><strong>9</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>137</strong></td>
<td><strong>54</strong></td>
<td><strong>83</strong></td>
</tr>
</tbody>
</table>
TABLE 3. Reactivity of unknown/suspect samples (n=227) in BpRAG1 and BPES ELISAs

<table>
<thead>
<tr>
<th>Category</th>
<th>Baylisascaris procyonis recombinant RAG1 antigen (BpRAG1) ELISA</th>
<th>Baylisascaris procyonis larval excretory-secretory antigen (BPES) ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Optical Density (OD)</td>
<td>Number of samples</td>
</tr>
<tr>
<td>Negative</td>
<td>&lt; 0.100</td>
<td>144</td>
</tr>
<tr>
<td>Probable negative</td>
<td>0.100 - 0.150</td>
<td>58</td>
</tr>
<tr>
<td>Suspect reactor</td>
<td>&gt; 0.150 - 0.200</td>
<td>16</td>
</tr>
<tr>
<td>Positive</td>
<td>&gt; 0.200</td>
<td>9</td>
</tr>
</tbody>
</table>