

T cell (CTL) killing of autologous B lymphocyte cell lines infected with a recombinant vaccinia virus expressing WNV E. T cell lines were generated by limiting dilution. We identified three novel human CD8 T cell epitopes contained within WNV E protein, restricted by HLA A*0201, HLA A68 and HLA B18, respectively. In a cohort of 19 HLA A2 individuals who received this vaccine, all developed WNV E A2 epitope-specific T cells detected by HLA tetramer staining. The frequency of CD3+CD8+ epitope-specific cells ranged from 0.1-2.6%. Peak tetramer frequencies were measured on day 14 in 5 (26%) and on day 28 in 14 (74%) of these individuals. We detected WNV E-specific CTL in 13 of 19 (68%). Peak CTL responses occurred on day 14 or 28 post-vaccination. Tetramer positive cells were detected at day 90, 180 and/or 360 in 15/17 (88%) of HLA A2 positive individuals for whom late samples were available. These data demonstrate that the YF backbone of this novel chimeric vaccine did not interfere with the development of robust CD8 T cell responses to the foreign gene (WNV E) in humans immunized with a novel chimeric virus vaccine. A West Nile virus vaccine that induces durable WNV-specific CD8 T cell responses as well as neutralizing antibodies is likely to be effective in protecting the host from disease.

1045

A RECOMBINANT WEST NILE SUBUNIT VACCINE PROVIDES EFFECTIVE PROTECTION AGAINST FATAL WEST NILE ENCEPHALITIS IN AGED AND WEANLING HAMSTERS

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Most human cases of West Nile disease are asymptomatic or result in a flu-like illness (West Nile fever), however, about 30-40% of cases reported to the Centers for Disease Control and Prevention in the last few years had severe neurological symptoms. Approximately 5-15% of the latter cases are fatal, and a high percentage of the non-fatal cases result in permanent neurological disabilities. Moreover, the fatality case rate is about 30% in victims over the age of 70. Currently, there is no approved commercially available vaccine for prevention of West Nile virus infection in humans, nor any specific therapy for disease. Hawaii Biotech has used a recombinant subunit approach for development of several flavivirus vaccines, including West Nile virus. The vaccines include envelope (WN-E) with or without non-structural (WN-NS) proteins produced in an insect cell expression system and purified by immunoaffinity chromatography. Low dose (1 mcg WN-E) vaccine formulations were found to completely protect (100% survival) golden hamsters against lethal West Nile encephalitis after challenge with live virus. Moreover, vaccinated animals remained free of clinical disease after challenge and had no detectable viremia. High titers of viral neutralizing and other antibodies were also elicited by the vaccine in hamsters (pre-challenge). Two major questions remained about the efficacy of a subunit vaccine approach for West Nile disease. One question was whether the vaccine would elicit a durable immune response. The other question was whether the vaccine would elicit a protective immune response in weanling and aged animal models. Protection (100% survival) against lethal viral challenge was demonstrated for at least 12 months beyond booster vaccination with a lack of viremia as well. These results show the vaccine to elicit a durable immune response. Viral neutralizing antibodies remained stable for this entire time. To answer the question of vaccine efficacy in young and old animals, both aged hamsters (12 months old at primary vaccination) and weanling hamsters (3 weeks old at primary vaccination) were shown to be completely protected against lethal challenge by low dose WN-E vaccination as well. This was shown with either of two different saponin-based adjuvants in the case of the aged animals. Viremia post challenge was reduced by 4-5 logs compared to adjuvant control aged animals, and high titers of antibodies were also produced (pre-challenge).

1046

INVESTIGATION INTO THE COMPARATIVE EFFICACY OF THREE WEST NILE VIRUS (WNV) VACCINES IN EXPERIMENTALLY INDUCED WNV CLINICAL DISEASE IN HORSES

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This is the first report of a comparative vaccine efficacy trial in horses where West Nile Virus (WNV) induced encephalomyelitis was demonstrated in all of the control animals. These studies demonstrate differences in the abilities of two commercial vaccines and a live chimera vaccine to protect against West Nile Virus induced disease at 28 days post-vaccination in horses. Since its emergence into the United States in 1999, West Nile Virus has killed a total number of 785 humans, 6,000 horses, and an estimated hundreds of thousands of birds. This mosquito-borne encephalitic flavivirus has caused clinical disease in 19,444 humans and 22,908 horses.² Using a reproducible model of WNV induced encephalomyelitis³, the efficacy of a new, live, chimera vaccine and two commercially available vaccines for prevention of clinical signs of West Nile virus (WNV) induced disease was tested in horses. Animals. Twenty-four healthy, WNV-seronegative horses of varying ages and gender, were randomized into 3 blinded vaccination/challenge trials. Horses were maintained according to University of Florida Animal Care Services and IACUC guidelines. Efficacy Trials. Horses were placed into three randomized, blinded trial groups consisting of 8 horses, with two horses in each group receiving: 1) a killed WNV vaccine, 2) a modified-live vaccine containing WNV prM and E proteins expressed by a canarypox vector, 3) a live chimera vaccine containing WNV prM and E proteins expressed by a YF17D vector, or 4) a diluent. For the commercial inactivated and modified-live vaccines, horses received an initial primary injection followed in 28 days with a second injection according to manufacturer's label. For the chimera vaccine, each horse received a primary injection dose of diluent followed in 28 days by a single vaccine dose. All horses were challenged intrathecally with virulent WNV 28 days after vaccination. Monitoring of Animals. Complete physical and neurological evaluations were performed for 21 days post challenge (PC). Horses were observed for clinical signs and neurological signs of mentation, fasciculations, paresis, ataxia, and cranial nerve abnormalities.⁴ Horses were euthanized for humane reasons due to overall severe health condition of the animal as a result of WNV infection and/or the inability to locomote without assistance. Twenty-one days PC, all remaining horses were euthanized and a full gross and histopathological evaluation was performed. Cross sections of the brain and spinal cord were examined and quantified for the presence of gliosis and perivascular cuffing. Serum and plasma samples were collected before and after challenge for virological evaluation. Statistical Analysis. Neurologic signs were analyzed as 0 = none and 1 = present. Data from all 3 trials were combined for X² and Fischer Exact analysis and clinical signs > 0 and present for > 1 day. Level of significance was set at P < 0.05. Statistical analysis was performed by use of computer spreadsheet (Excel, Microsoft, Redmond, WA) statistical analysis package (SysStat, Point Richmond, CA). Three of 6 control horses developed increased rectal temperature (> 102.5°C) and 6/6 developed PC viremia, grave neurological disease, and were euthanized for humane reasons before the end of the trial. Gross and histopathological changes consistent with WNV polioencephalomyelitis were observed in all control horses. None of the vaccinates, irrespective of the vaccine administered, developed PC viremia, and all survived to the end of the trial period, at which time a full gross and histopathological evaluation was performed. For the chimera group, none of the vaccinates developed increased rectal temperatures and no neurological signs were observed. One of the horses given the modified-live vaccine was determined to have pre-existing neutralizing antibody to WNV and was removed from the study. For the modified-live vaccinates, 1/5 horses developed increased rectal temperatures and 1/5 had consistent mild neurological signs in several categories. Another had mild malaise and anorexia after challenge. For

horses given the inactivated vaccine, 1/6 developed increased rectal temperature and 4/6 develop mild to moderate neurologic signs that occurred relatively late in the challenge period. Chimera vaccinates demonstrated significantly less clinical signs than horses vaccinated with the inactivated vaccine ($p \leq 0.035$) or control animals ($p \leq 0.01$). Histopathological analysis demonstrated moderate to severe changes in the controls consistent with WNV encephalitis and mild changes in some vaccinated horses. Using a severe challenge model of WNV that induced encephalomyelitis, the efficacy of a new, live, chimera vaccine and two commercially available vaccines for prevention of clinical signs of West Nile virus (WNV) induced disease was tested in horses. Challenge of horses by this model caused grave and reproducible neurological signs in all six horses that were not vaccinated. In contrast vaccination with one dose of the chimera vaccine or two doses of the commercial inactivated or modified-live vaccines resulted in 100% survivorship. Horses in both the inactivated and modified-live vaccine groups had a higher occurrence of clinical signs PC when compared to the chimera group. The efficacy of these vaccines against WNV induced disease in horses challenged >28 days post-vaccination is not known. Additional studies with larger sample sizes and at longer durations after initial vaccination are warranted.

1047

DISCOVERING NOVEL BLOOD STAGE MALARIA VACCINE CANDIDATES: SCREENING WITH IMMUNE SERA FROM FALCIPARUM MALARIA PATIENTS AND ASYMPTOMATIC PARASITE CARRIERS

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Completion of the reference genome sequence of *Plasmodium falciparum* has opened the way to discovery of novel malaria vaccine candidates. There are several lines of evidence from studies of naturally acquired immunity that immune response to the defined blood stage antigens is associated with protection against either malaria infection or clinical malaria, which makes the development of asexual blood stage vaccines feasible especially for residents in endemic areas. Here we took steps towards getting schizont-merozoite stage antigen proteins: creating customized and manageable set of genes for cloning according to the reports on transcriptome and proteome, and synthesizing recombinant protein from ~150 genes by a wheat germ cell-free system. These recombinant proteins were then screened for reactivity to immune sera from malaria exposed Thai individuals. The sera samples used are divided into two groups; from those hospitalized with the past history of falciparum malaria, or from the residents without clinical symptoms (asymptomatic parasite carriers) in continuously-monitored cohort village. The data processing after analysis of individual sera samples marked the difference between these two immune groups in the reaction pattern against a panel of recombinant proteins including known blood stage vaccine candidates. This screening approach, combined with high-throughput protein expression, will help to prioritize antigen molecules for detailed study in terms of protective potential and eventually lead to novel asexual blood stage vaccine candidates involved in humoral immunity.

1048

DISCOVERING NOVEL MALARIA PRE-ERYTHROCYTIC ANTIGENS

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Naval Medical Research Center is developing a multi-stage malaria vaccine designed to prevent malaria infection and to reduce clinical manifestations of disease, especially severe disease or death, in those who become infected. The feasibility of developing such a vaccine is supported by the sterile protection induced in humans by the radiation-attenuated sporozoite vaccine, which prevents parasites from fully developing in the liver, therefore preventing the onset of the erythrocytic infection. Although unknown, the antigens inducing this protective immune response are thought to be expressed in both the sporozoite and in the infected hepatocyte stages of the parasite. We have previously identified and cloned a panel of *P. falciparum* genes putatively expressed in the sporozoite and/or hepatocyte stages of the parasite. The high throughput expression of these genes as recombinant proteins was successfully achieved by a modified Wheat Germ cell-free system; this protein expression platform consistently yielded over 80% expression efficiency, with the majority of expressed proteins in soluble form. Several of these proteins were then chosen by batch screening for reactivity to panels of immune sera from naturally immune individuals and from volunteers immunized with irradiated sporozoites. Following affinity purification of these recombinant proteins, we raised polyclonal mouse and rabbit sera for antigen characterization studies such as parasite stage expression, cellular localization and functional activity. The analysis of several of these sera by immuno fluorescence (IFA) and Western Blotting assays have identified proteins expressed in a wide range of parasite stages including eight expressed in sporozoite stages, three in liver stages, four in various erythrocytic stages and three in gametocyte stages. These and future studies will help to qualify the potential of these antigens to serve as vaccine candidates. The combination of the Wheat Germ cell-free protein expression and screening protocols greatly enhances our ability to identify pre-erythrocytic stage vaccine candidates by their reactivity to antibody and more importantly to T-cell immune responses.

(ACMCIIP Abstract)

1049

CHIMERIC MSP-1 BASED VACCINE-INDUCED ANTIBODIES CROSS-REACT WITH SEVERAL PLASMODIUM SPECIES AND INDUCES PROTECTIVE IMMUNITY

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We have previously reported the design and use of linear polymeric synthetic peptide chimeras as viable delivery system for subunit malaria vaccines. Distinctive features of such synthetic chimeras are the inclusion of *Plasmodium* universal T cell epitope and their topology that facilitates extensive polymerization. To further assess the relevance of malaria universal T cell epitopes in vaccine design we constructed a synthetic gene that includes two universal T cell epitopes reported by us in *P. vivax* and located upstream from the *P. yoelii* MSP-1(19) fragment. The synthetic gene, codon optimized for expression in *E. coli*, also includes two different

tags used for protein purification and the assessment of antigenic integrity using monoclonal antibodies. Groups of BALB/c and C57BL/6 mice were used to test immunogenicity of the recombinant construct. A single immunization induces high antibody titers against both recombinant chimera and synthetic peptides. Specific antibody concentration for each isotype induced by immunization could be ranked as IgG1=IgG2a=IgG3>IgG2b for BALB/c mice and IgG2a>IgG1=IgG3>IgG2b for C57BL/6. These results suggest that different populations of T helper cells are induced after immunization with the recombinant construct. Protein and synthetic peptide-specific IFN- γ recall responses were identified in BALB/c mice after priming. This is in contrast with the predominant protein-specific IFN- γ recall response identified in C57BL/6. Interestingly, sera samples obtained from immune mice recognize recombinant proteins representing the two allelic forms of *P. falciparum* MSP-1(19) as well as native *P. falciparum* proteins on western blots. After experimental challenge mice were partially protected against infection and malarial anemia. Our results demonstrate the potential for developing complex recombinant chimeras containing parasite universal T cell epitopes as malaria vaccine candidates.

1050

TOLERABILITY AND IMMUNOGENICITY OF A *P. FALCIPARUM* MULTI-ANTIGEN MULTI-STAGE ADENOVIRUS VECTORED VACCINE, NAVAL MEDICAL RESEARCH CENTER-M3V-AD-PFCA, IN NZW RABBITS

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We have developed a candidate malaria vaccine, Naval Medical Research Center-M3V-Ad-PfCA, designed to induce cellular and humoral immune responses against both pre-erythrocytic and erythrocytic stages of the *P. falciparum* parasite life cycle. The vaccine is a cocktail of two Ad5 serotype adenovirus vectors (E1, E4, partial E3 deleted) expressing PfCSP and PfAMA-1. We tested Naval Medical Research Center-M3V-Ad-PfCA in a repeat-dose GLP study in NZW rabbits to evaluate its safety, toxicology and immunogenicity profile. The vaccine was administered at either the full anticipated human dose (1x10¹¹ pu in 1 mL) or a low dose (2x10¹⁰ pu in 1 mL) intramuscularly on study days 1, 11, and 32. Animals were evaluated for mortality, clinical observations, body weight, food consumption, ophthalmology, body temperature, dermal evaluation of injection sites, clinical pathology, gross pathology, organ weights, histopathology, and malaria-antigen specific immune responses. Immunogenicity was evaluated pre-study, 2 days post each vaccine administration, and at necropsy. Necropsy evaluations were 2 days and 14 days post-final vaccine administration. There was no apparent systemic toxicity. Occasional transient increases in body temperatures at 3 and 24 hours post vaccine administration returned to normal prior to the subsequent dose. Naval Medical Research Center-M3V-Ad-PfCA-dosed animals developed a minimal to mild erythema/edema and a minimal to mild inflammatory response at the injection site. Minor changes in globulin, cholesterol, triglycerides, and albumin to globulin ratio were considered part of the inflammatory response. These responses resolved and did not suggest clinically relevant adverse effects. There were no adverse effects on mortality, clinical observations, dermal injection site evaluation, body weights, food consumption, ophthalmologic findings, gross pathology or histopathology. PfCSP- and PfAMA-1- specific antibodies were detected in the rabbit sera, demonstrating that Naval Medical Research Center-M3V-Ad-PfCA was immunogenic. Overall, these data support the clinical evaluation of Naval Medical Research Center-M3V-Ad-PfCA.

1051

PRE-CLINICAL STUDIES TOWARDS RAD35-BASED MALARIA VACCINES

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Each year more than 1 million people succumb to malaria and therefore a safe and effective vaccine is a global health priority. Evidence thus far suggests that an effective vaccine against malaria should elicit potent antibody and T-cell responses, which adenoviral vectors in general are able to induce. However, clinical utility of commonly used rAd5-based vaccines may be hampered by wide spread pre-existing anti-Ad5 immunity in human population. Here we show that human adenovirus type 35 (rAd35) has low sero-prevalence worldwide including countries where malaria is endemic. We demonstrate that immunization with rAd35.CS vaccine resulted in strong T-cell responses against *P. yoelii* CS and protection upon high dose *Plasmodium yoelii* challenge in either naïve mice or mice carrying high level anti-Ad5 neutralising activity. Furthermore, we show that an rAd35.CS vaccine induced strong T-cell responses in macaques against the *P. falciparum* derived CS insert. Although clinical trials will eventually establish the utility of this vaccine, we further investigated the potency of a rAd35.CS vaccine in a near-physiological human skin model. Results of these experiments and data concerning further exploration of improved adenovirus based vaccines will be discussed.

(ACMCIP Abstract)

1052

IMMUNOGENICITY AND PROTECTIVE EFFICACY AGAINST PLASMODIUM VIVAX IN AOTUS MONKEYS FOLLOWING HETEROLOGOUS PRIME-BOOST IMMUNIZATION WITH PLASMIDS AND ADENOVIRUS VECTORS ENCODING PVAMA1 AND PVMSP1-42

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We have evaluated the immunogenicity and protective efficacy of heterologous DNA prime/adenovirus boost vaccine regimens in the *Aotus lemurinus lemurinus* malaria blood stage challenge model. Coding sequences for blood stage antigens PvMSP1-42 and PvAMA1 (Sall strain) were inserted into separate plasmid and adenoviral (Ad5) vectors. DNA alone, Ad5 alone, and DNA prime-Ad5 boost regimens were tested for the antigens in combination, and the DNA prime/Ad5 boost regimen for each antigen individually. For prime/boost regimens, three intradermal injections of DNA were administered at four week intervals at doses of 500 ug per plasmid (single-antigen groups) or 250 ug per plasmid (combined-antigen groups), followed fifteen weeks later by a single, intradermal Ad5 boost of 1e10 vp (viral particles) per construct (single antigen groups) or 5e9 vp per construct (combined antigen groups). The same regimens were used when the prime or the boost was administered alone, except that empty vectors were administered in place of the omitted immunization. Five weeks after the last immunization, all animals plus five unimmunized infectivity controls were challenged by intravenous administration of 10,000 parasitized erythrocytes in 1 mL RPMI 1640. Development of parasitemia was followed daily from days 1-35 post-challenge. The DNA prime/Ad5 boost regimens of PvAMA1 alone, PvMSP1-42 alone, or their combination, and, to a lesser extent, DNA prime by itself utilizing both antigens, protected the majority of animals, as measured by the ability to self-cure,

while Ad5 boost alone utilizing both antigens was not protective. The course of parasitemia, hematology, and humoral immune responses to the vaccine regimens as measured by ELISA using recombinant proteins and IFA against whole parasites, will be presented.

(ACMCIP Abstract)

1053

EFFECTIVE BOOSTING VECTORS FOR MALARIA IMMUNIZATION EVADE THE CD8 T CELL RESPONSE GENERATED BY PRIMING

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Prime-boost regimes are required for the induction of robust CD8 T cell responses by vaccination. Much descriptive work has been done to describe possible combinations of priming and boosting vectors. However little is known about the requirements for a robust recall response and, by extension, why some vectors boost and others fail. We tried boosting a *Plasmodium yoelii* sporozoite specific CD8 T cell response with recombinant flu, vaccinia and adenovirus vectors expressing the malaria circumsporozoite (CS) protein epitope SYVPSAEQI. Only recombinant vaccinia was able to effectively boost the immune response. The ability of vaccinia to boost was found to be independent of CD4 T cells but absolutely dependent on dendritic cells. We compared the abilities of the different vectors to prime naïve transgenic CD8 T cells specific for the CS epitope in the presence of pre-existing sporozoite immunity. Only vaccinia was able to overcome the preexisting immunity and prime the transgenic cells. In contrast flu and adenovirus vectors were unable to prime naïve cells in the presence of antigen specific memory CD8 T cells. This suggests that vaccinia is an effective boosting vector because it is able to carry antigen for presentation in the face of the memory CD8 response generated by priming.

(ACMCIP Abstract)

1054

NATURE BEATS NURTURE: A CASE STUDY OF THE QUALITY OF MALE ANOPHELES GAMBIAE S.L MOSQUITOES REARED IN ARTIFICIAL AND NATURAL ENVIRONMENTS.

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Laboratory experiments form the majority of our knowledge of the behaviour, physiology and ecology of many organisms; and in particular insects. However, the accuracy with which lab-derived estimates of insect life history and behaviour can predict their fitness and population dynamics in the wild is rarely validated. Such comparison is especially essential in cases where lab-derived information is used to formulate environmentally-conducive strategies for insect control. Here we conducted a comparative study of the reproductive potential and life-history of male *Anopheles gambiae* s.l mosquitoes under optimal lab conditions and at ambient conditions in an area of intense malaria transmission in East Africa. We measured three indirect indicators of male mosquito fitness: energetic reserves, body size, and survival, in a bid to understand if the demographics and energetic limitations of wild males can be correctly predicted from their lab counterparts. Crucially, we found that the body size and lipid stores of wild males were substantially greater than those from ideal lab conditions. These results infer that simplified lab environments underestimate the nutritional benefits that wild mosquitoes

can obtain from foraging freely. We caution that the energetic limitations of insects as identified in the lab may underestimate their resilience in the wild, and discuss the implications of this phenomenon with respect to malaria control programmes based on releasing genetically modified male mosquitoes.

1055

MALE ANOPHELES GAMBIAE MATING SUCCESS IN A SWARM: 'MAY THE BEST MAN LOSE'

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The use of genetically manipulated (GM) and /or sterile male mosquitoes for malaria control is becoming a possibility. The success of such programmes requires better understanding of male mating biology and ultimate mating competitiveness. It has been observed that factors such as body size and energetic reserves are reliable indices of mating success in female *Anopheles gambiae* mosquitoes and other flying insects. Here we investigated the influence of these traits on male mating success by manipulating larval nutrition. We hypothesized that male quality as indexed by nutrition-dependent body size and energetic reserves would be a reliable indicator of mating competitiveness in a swarm. Male *An. gambiae* larvae were reared at high, intermediate and low food conditions to generate adult males that differed in three typical indices of mating fitness: body size, energetic reserves, and survival. Resulting adult males were competed against one another for access to females. When competed against one another in swarms, males from the intermediate food treatment were 10 times more successful in obtaining females than those from the highest food treatment (Odds ratio [95% CI]=10.33 [2.1-19.52]), and 3 times more competitive than males from the low treatment (Odds ratio [95% CI] = 3.93 [0.93 - 4.71]). Body size, reserve provisioning and survival could not explain the success of the intermediate group. Instead we observed that males from the intermediate treatment were closest in body size to females, and hypothesize that phenotypic similarity of males to females is a more important predictor of their mating competitiveness than overall quality. This observation of assortative mating between *An. gambiae* of similar phenotypes has important implication for control programmes aiming to reduce malaria transmission by mass releasing sterile and /or genetically or phenotypically modified males. In order for released males to be competitive, they must be reared in backgrounds as similar as possible to wild-type females.

1056

DIFFERENTIAL SEGREGATION OF MATERNAL LIPIDS AS A STRATEGY FOR NEONATE LARVAE SURVIVAL IN THE MOSQUITO

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In animals, lipids are a source of energy, cell membrane components, signaling pathway modulators, and emulsifying agents. Because lipids serve functionally different purposes, segregation of lipids in appropriate tissues is essential for the birth of healthy neonates. We found a novel role of maternal lipids in newly hatched mosquito larvae. To deliver traceable lipids analogs into oocytes, we used purified lipophorin from adult *An. gambiae*. We found that the lipophorin delivers the lipids to the developing oocyte in an energy-dependent, receptor-mediated process. When oocytes from these mosquitoes were examined, fatty acids

and phospholipids were found to be co-localized within the same yolk bodies. To investigate how imported lipids are distributed in embryonic mosquito larvae, we collected eggs from mosquitoes that were injected with a mixture of phospholipids and fatty acids. When the eggs hatched, both the phospholipid and fatty acid analogs were found in the neonatal larvae. Distribution of the lipid types, however, was notable in these newly hatched larvae. A significant portion of the fatty acid segregated in spherical vesicles in the thorax, and along the side of the body cavity. This indicated that in addition to providing resources for biosynthesis of cellular components, a portion of fatty acids are stored along the body cavity and thorax for unknown purposes. In contrast, imported phospholipid was segregated differently inside the neonate intestine and in the gastric caeca. This unique segregation of the fatty acids and the phospholipids was reproducible in all repeated experiments. These observations indicate that newly hatched larvae need the maternal lipids to use even after emergence. We will discuss the implications this unusual segregation of maternal lipids has on the survival of newly hatched larvae. We will also discuss the implications these findings have to developing novel strategies of mosquito control, to reduce the burden of mosquito-borne diseases.

1057

DEFORESTATION AND ITS EFFECTS ON THE SPOROGENIC DEVELOPMENT OF *PLASMODIUM FALCIPARUM* IN *ANOPHELES GAMBIAE* IN THE HIGHLANDS OF WESTERN KENYA

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This study investigated the effects of microclimatic changes caused by deforestation on the sporogonic development of *Plasmodium falciparum* in *Anopheles gambiae* in an epidemic prone area of the western Kenya highlands. *A. gambiae* mosquitoes were fed with *P. falciparum* infected blood through membrane feeders. Fed mosquitoes were divided into different cages and placed in houses in forested and deforested areas in the village of Iguhu, Kakamega, western Kenya (elevation, 1500 m above sea level). Indoor and outdoor temperature and relative humidity in forested and deforested areas were measured by a hobo data logger. Indoor maximum and mean temperatures in the deforested area were higher than those in the forested area by 3.6°C and 1.2°C respectively. Average time for oocyst to appear in mosquitoes was 0.9 days shorter in mosquitoes placed in the deforested area than those in the forested area (7.5 vs. 8.4 days; $P < 0.0001$). The average time for *P. falciparum* to develop to sporozoites was 1.1 days shorter in the deforested area than that in the forested area (12.8 vs 13.9 days; $P < 0.05$). Infection rates of mosquitoes placed in the deforested area were similar to the forested area (22.6 % vs. 16.7%; $P > 0.05$). This study showed that deforestation increases the indoor temperature and consequently decreases the sporogonic time of *P. falciparum* in *A. gambiae*. Reduced sporogonic development time by deforestation enhances vectorial capacity of *A. gambiae* and thus increases malaria transmission risk.

1058

COMPLEXITY OF VERTEBRATE BLOOD UTILIZATION IN *ANOPHELES GAMBIAE*: ROLES OF PROTEASES AND LIPASES

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Adaptation to utilize vertebrate blood has provided access to highly nutritious meals for mosquitoes. Indeed, anautogenous mosquitoes require vertebrate blood for reproduction and propagation. However, the adaptation also poses the challenge of evolving specialized digestive

enzymes. Major enzymes of protein digestion are a number of trypsins and carboxydases. Trypsins are endopeptidases which cleave polypeptides internally at the C-terminus of only lysine and arginine residues. In contrast, carboxypeptidases are exopeptidases that non-specifically cleave from the C-terminus of any polypeptide. Vertebrate blood contains many diverse types of proteins with variable numbers of lysine and arginine residues, potentially resulting in differential kinetics of protein degradation. In addition, lipid-protein complexes in vertebrate blood, VLDL, LDL and HDL, with the kinetics of expression of diverse proteases and lipases, make vertebrate blood digestion a complex process. To gain a deeper insight into the initial stages of the blood digestion process, we have utilized density ultracentrifugation, specific mono and polyclonal antibodies, and protein digestion analysis *in vitro* and *in vivo*. We have also used the kinetics of lipid release from lipid-protein complexes after the density ultracentrifugation of human and mouse blood components. The study has provided a comprehensive look at the different dynamics of individual components of vertebrate blood in the midgut of the malaria vector with which the developing pathogens compete.

1059

SPECIATION BY ECOTYPIFICATION IN *ANOPHELES GAMBIAE*: A QUANTITATIVE TEST

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In his ecotypification hypothesis, M. Coluzzi describes how paracentric inversions may lead to Anopheline speciation through suppressed recombination. He suggests that suppressed recombination could prevent the homogenization of co adapted loci with maladapted alleles. His verbal model is supported by recent discoveries of genes under selection within inversions and in other areas of reduced recombination, but it has not previously been subjected to a quantitative test. The large body of work on *Anopheles gambiae* s.s. and new theoretical results make it possible to evaluate the likelihood of speciation by ecotypification using a stochastic model. We created a simulation that captures the essential properties of the ecotypification process, validated it against independent theoretical expectations and performed systematized experiments. In addition to testing overall probability that ecotypification may lead to speciation, analysis of the experimental results shows which factors are most important for inversion polymorphism. Our study of this synthetic system is offered as a guide to future field and laboratory investigations of speciation in *An. gambiae* and related taxa.

1060

FEEDING AND RESTING BEHAVIOR OF *ANOPHELES LONGIPALPIS* (THEOBALD) IN AN AREA OF HYPERENDEMIC MALARIA TRANSMISSION IN SOUTHERN ZAMBIA

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Anopheles longipalpis (Theobald) (Diptera: Culicidae) is a predominantly zoophilic mosquito that has not yet been implicated in malaria transmission. However, this species was collected indoors with *An. funestus* s.l. in southern Zambia where transmission of *P. falciparum* is hyperendemic, was confused morphologically and molecularly with *An. funestus* s.l., and has been reported to feed on humans. Our objective was to define the actual or potential role of *An. longipalpis* in transmission of *P. falciparum* in Mufwafwi village in southern Zambia where large numbers of indoor resting, engorged specimens were collected. The resting density, blood feeding behavior and human biting rate of *An. longipalpis* was investigated during the 2004-2005 and 2005-2006 transmission seasons. Numbers of *An. longipalpis* peaked towards the end of the rainy season.

Although 6 specimens were collected during human landing catches, the feeding behavior of this species was significantly biased towards cattle (88.7%), with other blood meals originating from dogs (7.6%) and goats (3.8%). Zero specimens of *An. longipalpis* were infected with *P. falciparum* based on PCR assays. Therefore, *An. longipalpis* was not confirmed to be involved in malaria transmission, although more extensive screening is needed. Correct morphological and molecular identification of this species is crucial for malaria control programs in areas where *An. funestus* s.l. and *An. longipalpis* exist sympatrically so that scarce resources are not wasted on the control of an apparent non-vector.

1061

INCREASED DETECTION RATE OF LEPROSY (HANSEN'S DISEASE) AND STRATEGY FOR DISEASE CONTROL IN RIO GRANDE DO NORTE, BRAZIL

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Hansen's disease (HD) remains an important public health problem in Brazil, where 38,410 new cases were detected in 2005, with the prevalence rate of 1.48 cases for 10,000 people, larger than the incidence recommended by the World Health Organization for elimination of the disease. HD has a focal distribution in Brazil and the prevalence varies by region. The Northeast Region has the third largest new case detection rate (NCDR) in the country (3.07 new cases per 10,000 people); however the state of Rio Grande do Norte State has one of the lowest NCDR in the Northeast. Careful analysis on reportable data between 1996 and 2005 showed that NCDR has rapidly increased; from 0.64 in 1996 to 1.60 new cases per 10,000 people in 2005. We present here data from a case-control study conducted in Rio Grande do Norte, Brazil. Geographic mapping of the HD cases, residential census, examination of HD cases and household contacts and genotyping of *Mycobacterium leprae* in Rio Grande do Norte were performed. A total of 258 households were visited with 724 of the 808 (89.6%) contacted subjects giving consent to participate in the study. Fifty seven of 724 (7.9%) people examined had skin or nerve findings suspicious for HD. Fifteen new cases of HD were confirmed, giving a detection rate of 2 new cases per 100 people examined. New HD cases were diagnosed by clinical and bacillary findings according to the Ridley-Jopling classification. All cases were started on WHO multi-drug treatment regimens for HD. The georeference of the HD cases using GPS showed that 76.8% of the cases detected lived in areas where the demographic density was high. The results of the case-finding activities show evidence for continued attention to HD case finding and the need to start early treatment. The observation of families with multi HD cases shows the need for studies to clarify the role of human genetic susceptibility and immune responses to *M. leprae* in determining progression to disease development.

1062

THE RISK OF LEPROSY IN INDIVIDUALS WITH A LOW AND HIGH HOUSEHOLD SOCIO-ECONOMIC STATUS IN NORTHERN BANGLADESH

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Population studies show that an increase of socio-economic circumstances leads to a decrease of leprosy incidence. The relation between socio-

economic status (SES) and leprosy at an individual level is uncertain. This study was conducted to determine the risk of leprosy for individuals with a high and low household SES. We conducted a case-control study in Bangladesh. Fifty leprosy cases were sampled from the study population of a prospective study on household contacts, and 100 controls were sampled from a multi-stage cluster sample of the general population. The household SES of cases and controls was determined using an asset index. Principal components analysis was used to determine weights for the individual assets. The relation between household SES and leprosy prevalence was determined through logistic regression modelling. The odds ratio between households with a low SES and a high SES was 3.0 (95% CI 1.33 - 6.79), indicating a three-fold increased risk of leprosy in poor households compared to rich households. In conclusion, the asset index is a practical tool to describe the SES of households. The increased prevalence of leprosy in households with a low SES may possibly be used to target control activities more effectively.

1063

COLEP: A CLUSTER RANDOMISED CONTROLLED TRIAL WITH SINGLE DOSE OF RIFAMPICIN TO PREVENT LEPROSY AMONG CLOSE CONTACTS OF NEWLY DIAGNOSED LEPROSY PATIENTS IN BANGLADESH

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Close contacts of leprosy patients are at an increased risk of developing clinical leprosy, the disease caused by infection with *Mycobacterium leprae*. In the past it was shown that prophylaxis with dapson could prevent leprosy among contacts, but this had to be administered over a long period of time. Rifampicin is a strong bactericidal drug against *M. leprae* and more effective than dapson. Uncontrolled or unblinded studies have shown that it is effective when used as a prophylactic drug against leprosy.

We performed a single-centre, double blind, cluster-randomised, placebo-controlled trial among 21,711 close contacts of 1037 newly diagnosed leprosy patients in northern Bangladesh. The intervention consisted of a single dose of rifampicin prophylaxis or placebo given to all contacts in the second month after the treatment of the patient had started. 19,956 contacts (91.2%) were examined for leprosy during the first follow-up after two years. The reduction in incidence after two years by a single dose of rifampicin was nearly 50% and the number needed to treat to prevent a single case of leprosy among contacts was 287. During the presentation the trial, its outcomes and further details with regard to specific risk groups will be presented. It will be discussed whether rifampicin prophylaxis is useful additional tool in leprosy control, which contacts would benefit most and operational issues which need to be taken into account when introducing prophylaxis in field programs.

1064

PREVENTION OF LEPROSY USING RIFAMPICIN AS CHEMOPROPHYLAXIS: RESULTS AFTER 6 YEARS FOLLOW-UP

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In 2000 a controlled chemoprophylactic intervention study started on five Indonesian island highly endemic for leprosy (4739 inhabitants in total) to determine whether rifampicin can be used as chemoprophylaxis to prevent leprosy. The population was actively screened before the intervention and subsequently once yearly for 6 years. On the control island no

chemoprophylaxis was given. On the largest island chemoprophylaxis was only given to household and neighbour contacts of leprosy patients ('contact' group) and on three small islands to all eligible persons ('blanket' group). The prophylactic regimen consisted of two times 600 mg rifampicin for adults and 300 mg for children (5-14 years) with four months between doses. At all islands the leprosy patients received regular MDT treatment.

The total cohort consisted of 3,964 persons, initially free of leprosy. After three years follow-up the yearly incidence rate in the control group was 39/10,000; the cumulative incidence was significantly lower in the blanket group ($p=0.031$). No difference was found between the contact and the control groups ($p=0.93$). To see whether this apparent reduced leprosy incidence in the first three years in the blanket group was due to a delayed development of leprosy or a complete clearance of infection the cohort was followed for another three years. The initial lowering effect of the chemoprophylaxis in the blanket group on the incidence of leprosy in the first three years was not seen in the second three years of the study; data analysis is currently ongoing. More results will be presented during the congress.

1065

ISOLATION AND CHARACTERIZATION OF *BARTONELLA BACILLIFORMIS* FROM AN EXPATRIATE ECUADORIAN

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An atypical *Bartonella bacilliformis* infection was diagnosed in an expatriate patient with chronic keloid-like skin lesions and splenomegaly three years after visiting Ecuador. Initial serology, PCR, and immunohistochemistry ruled out *B. henselae* and *B. quintana*, but pathology of the splenic biopsy was suggestive of bacillary angiomatosis. Bacilli subsequently isolated from the patient's blood were identified as *B. bacilliformis*. EC01 was isolated from blood after 18 days incubation on 5% rabbit blood-heart infusion agar with phosphate buffer overlay at 28°C. DNA was analyzed by nested PCR using a panel of *Bartonella* gene primers, protein banding patterns were determined by 1-D gel electrophoresis and Western blot, and EC01 antigens were tested by immunofluorescence assay (IFA) against a panel of antisera from patients with Oroya fever in Peru. This case was unusual as the patient was inapparently infected with *B. bacilliformis* in a nonendemic area of Ecuador and clinical signs of illness were greatly delayed and atypical for classical bartonellosis. The EC01 gene sequences (*gltA* and *ITS*) and protein band banding pattern were most similar to a sub-set of *B. bacilliformis* isolates from the endemic region of Caraz, Ancash in Peru. By IFA, patient serum did not react with all Peruvian *Bb* isolates tested nor did EC01 antigen react with all Oroya fever sera. Hyperimmune rabbit antisera to *B. bacilliformis* bound to similar proteins of EC01 and other isolates of *B. bacilliformis* from Peru by Western blot. Electron microscopy also showed the presence of peritrichous flagella on EC01. In conclusion, infections with *B. bacilliformis* may present with unusual clinical signs and be missed by standard diagnostic tests, so it is important to attempt isolation. The true distribution of *B. bacilliformis* in South America, as well as patient and microbial factors which contribute to atypical symptoms of infection, are poorly understood.

1066

IMMUNOLOGICAL PATTERN OF PATIENTS WITH ACUTE AND CHRONIC PHASE OF *BARTONELLA BACILLIFORMIS* INFECTION IN A ENDEMIC AREA IN PERU

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Human Bartonellosis is produced by *Bartonella bacilliformis*, a flagellated gram negative bacterium, and may have two clinical presentations. The acute phase is characterized by a feature of sepsis with RBC's parasitemia, or a chronic phase characterized by cutaneous lesions. This is the first study that evaluates the immunology patterns of patients in endemic area. It was a transversal pilot study that included patients in two hospitals in Ancash-Peru, and one hospital in Lima-Peru. Patients between 5 and 60 year-old, with acute or chronic phase according standardized Peruvian criteria were included. Pregnancy, previous immunosuppression, or recently antibiotics use were exclusion criteria. T CD4+, and CD8+ cells recount cells was done by FACSCalibur flux cytometer. INF- γ , IL-2, TNF- α , IL-4, IL-10 measured were by BD OptEIA™ ELISA Kits. Data were analyzed using SPSS 11v. Variables were transformed to Log10 for ANOVA one-way and Tukey Pairwise comparisons. With previous consent, 9 people in the control group, 21 patients with acute phase, and 17 with chronic phase were included. The ANOVA-one way analysis showed differences among groups for INF- γ ($p<0.005$) and IL-10 ($p<0.005$). In the acute phase, the mean of CD4+ was 866.5, CD8+ 829.5, INF- γ 152.34, IL-10 99.39 (SD 121.45). In chronic phase, the mean of CD4+ was 808.12, CD8+ 714.94, INF- γ 23.69, IL-10 23.36 (SD 27.99). In Tukey analysis, for INF- γ and IL-10, there were statistic difference between the acute and chronic phase group ($p<0.001$), and between the acute and control groups ($p<0.001$) only for IL-10. Using binomial probability, in the acute phase, there was a significant difference between expected and observed CD4+ ($p<0.001$) and CD8+ outliers ($p<0.001$), with 6/10 and 5/6 outliers over normal range respectively; also, in the chronic phase in CD4+ ($p<0.005$) and CD8+ ($p<0.001$), with all outliers over normal range. In conclusion, CD4+ and CD8+ T cells recount were abnormal in acute and chronic phase, mainly over normal limits, however, some patients during the acute phase had values below normal range, that may explain infectious complications. Significant high level of IL-10 was found in acute phase. In gram negative sepsis, an uncontrolled production of IL-10 may produce an "immunological paralysis" of antigen presenting cells. This phenomenon may explain severe courses in some patients, and deserves further study.

(ACMCIP Abstract)

1067

THE IDENTIFICATION OF *IN VIVO* INDUCED PROTEIN ANTIGENS DURING *BACILLUS ANTHRACIS* INFECTION

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In vivo induced antigen technology (IVIAT) identifies immunogenic bacterial genes expressed specifically during infection and not during in

in vitro culturing. Convalescent serum from patients or animals infected with a pathogen of interest are pooled and extensively adsorbed against the cognate pathogen grown under standard laboratory culture conditions. This adsorption step removes antibodies that bind antigens expressed during *in vitro* growth, while retaining antibodies that recognize antigens specifically expressed during infection. Adsorbed serum is then used to probe a protein expression library. We applied IVIAT to *Bacillus anthracis*, the cause of anthrax. We pooled convalescent sera from immunized macaques surviving inhalational challenge with fully virulent Ames strain *B. anthracis* spores. We adsorbed this pooled sera against *B. anthracis* organisms grown in BHI broth in air. We then used the adsorbed serum to probe an inducible 125,000 clone *B. anthracis* protein expression library established in *Escherichia coli*. We identified twenty *B. anthracis* antigens as immuno-reactive using IVIAT, including PagA (protective antigen; supporting the validity of the screen), and six members of a N-acetylmuramoyl-L-alanine amidase (NALAA) family. Using quantitative real time PCR comparing RNA isolated from *in vitro* cultured cells to RNA isolated from BALB/c mice infected with virulent Ames strain, we confirmed induced expression *in vivo* for a subset of *B. anthracis* genes identified by IVIAT, including *pagA*, NALAA *amiA* (pXO2-42), BA3767 and *plyL* (BA4073), and a putative bacteriophage holin gene, BA4074 (possibly co-transcribed with BA4073). We have generated histidine-tagged fusion proteins for two *B. anthracis* antigens identified by IVIAT, and confirmed a specific immune response post-wild-type *B. anthracis* challenge in a subset of macaques for NALAA BA3737. The identification of immunogenic *B. anthracis* proteins expressed *in vivo* during anthrax could have diagnostic, therapeutic or preventative implications for this zoonotic infection.

1068

THE IMPACT OF SCHISTOSOMIASIS AND INTESTINAL HELMINTH CONTROL PROGRAM ON HEALTH IN RURAL UGANDA

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Two rounds of treatment with praziquantel and albendazole have been delivered in Uganda to high risk communities, and monitored annually by following a cohort of children before and after treatments. 1871 children were successfully examined on three annual visits. There was evidence that mass treatment with praziquantel and albendazole lead to a significant reduction in intensity of *Schistosoma mansoni* (70% (95% Confidence Interval [CI] 66-73%) after one year and 82% (95% CI: 80-85%) after two years). Intensity of hookworm infection was also reduced (75% and 93% - unadjusted). There was also a significant increase in haemoglobin concentration (0.135 g/dL (95% CI: 0.126-0.144), after one year and 0.303 g/dL (95% CI: 0.293-0.312) after two years), and significant reductions in signs of early clinical morbidity. The impact of intervention on *S. mansoni* levels was similar to that predicted by mathematical models of the impact of chemotherapy for human schistosomiasis. Improvements in haemoglobin were greatest for those children found to be anaemic or harbouring a heavy *S. mansoni* infection at baseline. In conclusion, anthelmintic treatment delivered as part of a national helminth control programme can reduce infection and morbidity due to schistosomiasis and intestinal helminths and can improve haemoglobin concentrations of schoolchildren.

1069

MONITORING URINARY SCHISTOSOMIASIS INFECTION IN COMMUNITIES GIVE A PRAZIQUANTEL 'HOLIDAY' AFTER FIVE ROUNDS OF TREATMENT

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Mass drug distribution of praziquantel (PZQ) at a dose of 40 mg/kg every 1-3 years can significantly reduce schistosomiasis morbidity in areas of high endemicity. However, the optimal interval for such treatment is debated. PZQ, which is not donated by pharmaceutical companies to National Control Programs, costs about US \$0.08 per 600 mg tablet. Therefore, drug costs in mass treatment programs can become substantial, and economizing through drug PZQ 'holidays' could allow more people to be treated by programs using drug rotation schemes through different endemic areas. In the local government area (LGA) of Pankshin in Plateau State, Nigeria, PZQ treatment for urinary schistosomiasis was launched in 1999. PZQ was administered in schools in communities where a sample of 30 children aged 10-14 were found to have a hematuria prevalence by dipstick of $\geq 20\%$ - $< 50\%$. In communities with higher prevalence ($\geq 50\%$ hematuria) PZQ was administered community wide (e.g., including adults). In eight sentinel villages in the LGA (4 receiving school-based treatment and 4 receiving community-wide treatment) we observed a dramatic decline in hematuria over a four-year period from a baseline mean of 40% (village range 30%-77%) hematuria prevalence among 240 children in 1999 compared to a mean of 5% (range 0-27%) among a independent group of 240 just prior to the fifth PZQ treatment administration in 2003. In consultation with the ministry of health of Nigeria, PZQ treatments were stopped after the fifth dose, while simultaneously intensifying a schistosomiasis health education campaign throughout the LGA. Two years after stopping PZQ mass treatments, in 2005, we again evaluated 240 children in the eight sentinel villages to look for evidence of recrudescence. We found the 2% hematuria rate (range 0-7%) to be essentially unchanged from 2003. We concluded that recrudescence had not occurred after a 2 year 'drug holiday' interval and a three year rotation would be 'safe.'

1070

HELMINTH INFECTIONS AND POLYPARASITISM AS PREDICTORS OF COGNITIVE PERFORMANCE OVER 18-MONTHS OF FOLLOW-UP AMONG SCHOOL-AGE CHILDREN

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The objective of this study was to estimate the effect of *Schistosoma japonicum* reinfection and changes in the burden of soil transmitted helminth (STH) infections on cognitive test performance. Longitudinal treatment-reinfection study with treatment for *S. japonicum* infection. Four tests were administered including: learning and memory domains of the wide range assessment of learning and memory (WRAML), verbal fluency and the Philippine nonverbal intelligence test (PNIT). Infection burden were determined for STH and *S. japonicum* and used to define infection intensity based. A dichotomous variable denoting: low reinfection burden vs. no change/higher reinfection, was defined for each species. Repeated measures multiple regression analysis was conducted

to assess the impact of changes in infections on test scores. None/low *S. japonicum* reinfection was predictive of high scores in the PNIT ($=1.05$, p -value= 0.016) and WRAML learning ($=2.33$, p -value= 0.014). Ascaris ($=4.10$, p -value= 0.028) and trichuris ($=4.17$, p -value= 0.016) intensity reduction were predictive of higher longitudinal performance in WRAML learning and WRAML memory respectively. Baseline number of STH infections was associated with lower scores in WRAML learning ($= -2.49$, p -value= 0.052) and PNIT ($=1.03$, p -value= 0.058) and decline in polyparasitic STH infections was predictive of higher performance in WRAML learning for girls ($=4.13$, p -value= 0.023). In conclusion, low *S. japonicum* reinfection, longitudinal decline in ascaris and trichuris intensity, and reduction of polyparasitic STH infections were predictive of higher performance in 3 of 4 cognitive tests suggesting that simultaneous control of *S. japonicum* and STH infections could improve children's ability to take advantage of educational opportunities in helminth endemic regions.

1071

MODELING SCHISTOSOMIASIS TRANSMISSION IN A DISTRIBUTED ENVIRONMENT: IMPLICATIONS FOR SUSTAINABLE CONTROL

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Social interaction and physical interconnections between villages can influence the spread of infectious agents. Using a mathematical model of schistosomiasis transmission for a distributed set of heterogeneous villages, we explored the effect of two forms of connectivity on disease transmission and control. One form of connectivity, cercarial and miracidial transport, occurs via hydrological channels that link adjacent villages. Another form of connectivity, social behavior, such as migrant labor, can lead to infection of laborers outside of their own villages, and potentially the spread parasite eggs to other villages. We modeled 15 hypothetical connected villages, each with differing potential to sustain transmission. We show that the two forms of connectivity can have important consequences for disease control. First, transmission can be sustained regionally throughout a group of connected villages even when individual village conditions would appear to not support endemicity (Basic Reproductive Numbers for all individual villages are less than one). Second, certain levels of connectivity lead to optimum transmission, which somewhat surprisingly, does not necessarily coincide with the largest spread of social contacts. Third, the targeting of villages with high snail and human infection, without regard to village interconnections may not lead to sustainable control. Analyses of distributed models may provide valuable insight into more sustainable control for a variety of parasitic diseases.

1072

SOCIO-ECOLOGY OF MALARIA AND URINARY SCHISTOSOMIASIS IN COASTAL KENYA

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Schistosomiasis and malaria are "diseases of poverty" and are co-endemic in many parts of the world. Despite the high global prevalence of these diseases few studies have examined the potential effects of co-infection on morbidity. Additionally, these diseases may share common upstream socio-economic or environmental risk factors. An improved understanding of the socioeconomic and ecologic context for such multiple infections may help focus prevention and control efforts. Accordingly, we undertook

a study to examine the contextual determinants of polyparasitism in coastal Kenya, with a focus on *Plasmodium* and *Schistosoma* infections. Following informed consent, 1300 eligible participants age 8 and above in Kingwede, Kenya were recruited to participate during April and May, 2006. Presence and intensity of *S. haematobium* infection was determined using standard urine filtration examination. Presence and intensity of *P. falciparum* infection was determined using standard thick and thin blood slide examination as well as by PCR. Hemoglobin, height and weight measures were also taken for children to determine anemia and stunting (via Z-scores). Participants were administered a detailed questionnaire in Kiswahili addressing schistosomiasis and malaria knowledge, self-reported water use and other health practices, as well as socioeconomic status (SES). The importance of these factors was assessed using regression models compared to marginal models that incorporate correlation of individuals within households. We also examined spatial patterns of schistosomiasis and malaria cases, determining environmental risk factors based on cluster analyses. Standard and multilevel analyses revealed important household level socio-economic risk profiles related to health knowledge and behaviors (e.g. water contact patterns, use of treated bed nets) that appeared to affect risk.

1073

TOWARDS INTEGRATED CONTROL TO ACHIEVE TERMINATION OF SCHISTOSOMIASIS TRANSMISSION IN IRRIGATED AGRICULTURAL REGIONS OF CHINA

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In the aftermath of the SARS outbreak in 2003, the Chinese government launched campaigns against a number of major communicable diseases including the parasitic disease schistosomiasis. To beef up the control, the State Council of China recently issued an unusual specific statute on schistosomiasis control calling for a comprehensive approach. Two endemic mountainous provinces with typical irrigated agriculture, Sichuan and Yunnan, were chosen to pilot aggressive control efforts and declared ambitious goals of effectively controlling schistosomiasis by 2008 and eliminating the transmission throughout the provinces by 2015. Here we report on village-level studies in Sichuan of the determinants of transmission and efforts required to effectively control even eliminate the disease transmission using a mathematical model. The model specifically incorporates time-varying environmental variables (e.g. water contact and temperature- and rainfall-driven events) which are believed to play important roles governing the transmission cycle. A new metric of transmission potential, environmentally-mediated R_0 , or basic reproductive ratio, arose from the study. The model was calibrated to field data and the calibrated model utilized to explore a range of competing control strategies. The results offer evidence of inadequacy of the niclosamide-praziquantel (mollusciciding-chemotherapy) strategy to achieve broadly sustainable intervention of transmission in this environment. It suggests that the goal of eliminating transmission will require environmental modifications and/or improved sanitation facilities, for example, alternation of village systems to permanently destroy snail habitat or better waste management to control parasite eggs, which permanently change the disease transmission potential, in addition to the continued use of praziquantel and niclosamide. The study has important policy implications for the ongoing schistosomiasis control in China.

1074

SPATIAL DISTRIBUTION OF URINARY SCHISTOSOMIASIS INFECTION AMONG SCHOOL CHILDREN IN AN ENDEMIC COMMUNITY IN GHANA

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Schistosoma haematobium infection is one of the major contributors to the disease burden in Ghana, after malaria. Careful detection of high transmission epicenters may offer the potential for a more effective, highly-focal snail control in conjunction with targeted chemotherapy to reduce transmission. In the present study, the Geographic Information System (GIS) was applied to incorporate demographic, parasitological, and household location data for school children in an endemic village, in the Cape Coast Municipality in Ghana. The prevalence of infection was 32.1% with the highest infection recorded among children in the age group 12-14. More males (33.7%) were infected compared to females (29.4%) and this is likely a result of differences in social and religious practices. However, there was no evidence of significance between them ($\chi^2 = 0.142$, $P = 0.71$, $CI = 95\%$). A strong positive correlation was found between water contact activity and infection ($\chi^2 = 27.164$, $P < 0.001$, $CI = 95\%$). GIS techniques were utilized for producing maps and analyzing the results. High infection intensities were clustered in the community and around different water contact sites. Though these results are preliminary, it confirms the small-scale focalization of the disease and shows that the GIS can be an important tool for schistosomiasis control, especially in areas where the introduction of alternative water sources and the implementation of mass community chemotherapy have failed to halt the continuing cycle of urinary schistosomiasis transmission.

1075

DISTRIBUTION OF FREE BEDNETS BUNDLED WITH INSECTICIDE VIA AN INTEGRATED CHILD HEALTH CAMPAIGN --- LINDI REGION, TANZANIA, 2005

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Use of insecticide-treated bednets (ITNs), a proven intervention to prevent malaria mortality, continues to be low, and new distribution strategies are needed. From July 30 to August 1, 2005, the Tanzanian Ministry of Health and UNICEF conducted an integrated health campaign with free distribution of untreated bednets bundled with insecticide, measles vaccination, vitamin A and mebendazole for children <5 years old (under-5s) in Lindi Region, Tanzania. Only written and illustrated instructions on treatment and use of bednets were provided. A community-based cross-sectional cluster survey to assess intervention coverage was conducted from November 2-16, 2005 during low-intensity malaria transmission season. Thirty enumeration areas (EAs) were selected using probability proportional to estimated size. Households in each EA were mapped, and twenty households per EA were randomly selected. Altogether, 574 households with 354 under-5s were visited. Most under-5s (79.6%) received a bednet. Because of the campaign, household possession of any bednet increased from 52.9% to 69.3% ($p < 0.001$) and possession of an ITN increased from 13.3% to 24.7% ($p < 0.001$). The distribution was equitable, and possession of bednets and ITNs increased in all wealth quintiles. Among households that had received at least one bednet, 99.4% reported retaining all campaign bednets. Caretakers reported that 46.3% of under-5s slept under a bednet, and 21.5% of under-5s slept under an ITN the previous night. The total cost per bednet distributed was \$2.47 (\$2.18 per bednet with insecticide and \$0.29 for distribution).

Integrating malaria prevention activities with immunization campaigns can rapidly and equitably increase possession and use of bednets and merits continued large-scale implementation. Rates of home treatment of bednets with insecticide were low, thus future distribution campaigns should provide factory-treated long-lasting ITNs. Use of ITNs by under-5s was low, and further work is needed to increase ITN use after distribution campaigns.

1076

DISTINCT *P. VIVAX* POPULATIONS IN MEXICO DIFFERENTIALLY INFECT TWO LOCAL VECTORS

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Two circumsporozoite (CS) protein repeat types (VK210/VK247) have been observed in *Plasmodium vivax* in southern Mexico, and their distribution approximates that of the two local vectors - *Anopheles pseudopunctipennis* and *An. albimanus*. Additionally, they have been shown to be associated with differential infectivity of the two vectors. However, the destruction of VK247 parasites in *An. albimanus* occurs prior to the expression of the CS protein. To further investigate the molecular basis of this host-specificity, and to better characterize the parasite population, we genotyped *P. vivax* isolates using 29 genome-wide microsatellites and 23 minisatellites flanking the *csp* gene and spanning 200 Kb. Using a model-based structuring method, we found support for multiple parasite sub-populations that in turn formed two distinct groups, which were supported by bootstrap analysis, showed little evidence of recombination, and differentially infected *An. albimanus* and *An. pseudopunctipennis*. The VK210 repeat type was found in both populations. The *csp* locus showed strong linkage disequilibrium in the *An. pseudopunctipennis* parasite population but not the *An. albimanus* parasite population, leaving open the possibility that one or more genes at this locus contribute to the infectivity phenotype in *An. pseudopunctipennis*.

(ACMCI Abstract)

1077

STRAIN- AND SPECIES-SPECIFIC COMPARISON OF THE IMMUNE RESPONSES OF DIFFERENT MEMBERS OF THE *ANOPHELES GAMBIAE* COMPLEX TO *PLASMODIUM FALCIPARUM* INFECTION

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Previous research has indicated that the susceptibility of *Anopheles gambiae* to *Plasmodium* infection varies according to the strain of mosquito and species of malaria parasite, indicating the existence and importance of strain- and species-specific variation in the outcome of mosquito infection with different malaria parasites. However, most laboratory studies are undertaken using only one, or several, mosquito strains and a single malaria species (usually the rodent malaria *P. berghei*) with the assumption that the results are generalizable to other mosquito-malaria parasite combinations. Consequently, we have undertaken global transcriptomic comparisons of naive and *P. falciparum*-infected adult female mosquitoes from different strains and species within the *An. gambiae* complex using whole genome oligonucleotide microarrays. We have identified a number of constitutive and *P. falciparum*-induced differences in gene expression between the different strains and species within the *An. gambiae* complex which we have functionally characterized using RNAi screens, and compared to *P. berghei* infection in the same mosquito strains/species. Although some aspects of the mosquito response to *Plasmodium* infection were conserved across mosquito-malaria

parasite combinations, our findings highlight the importance of strain- and species-specific differences in the immune responses of different members of the *An. gambiae* complex to infection with different *Plasmodium* species.

(ACMCIP Abstract)

1078

DRY SEASON MALARIA TRANSMISSION IN A RURAL SUDAN SAVANA OF MALI

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Malaria transmission is considerably reduced during the dry season. In the village of Bancoumana, while adult mosquitoes were barely detectable, studies have shown that new *Plasmodium falciparum* infection occurred during the dry season. The aim of this study was to identify potential sources of *P. falciparum* infection during the dry season and provide basis for selective control strategy in Mali. From December 2004 to May 2005, we carried out a monthly active search of breeding sites and adult mosquitoes collection in Bancoumana village and in a 5 kilometer distant fishing hamlet (Bozokin) lying along the Niger River. Mosquitoes were collected by pyrethrum spray catch methods. Vector infection rate and molecular forms were determinant by ELISA and PCR methods respectively. Passive surveillance was performed to record all microscopically confirmed cases of malaria in children of 0-15 years seeking for treatment at the health center of Bancoumana. Among *Anopheles gambiae* complex, *An. arabiensis* represented 11.4% (17/149) in Bancoumana and only 2.1% (8/374) in the hamlet. The M molecular form of *An. gambiae* s.s. was the most abundant in both localities. However its frequency in Bancoumana (92.6%, n = 95) was significantly higher (X₂ = 4.51, P = 0.034) than in the fisher hamlet (84.0%, n = 319). The mosquito monthly biting rates were 0.63 (Minimum = 0.04, Maximum = 1.3) in Bancoumana and 35.4 (Minimum = 15.4, Maximum = 82.1) in the hamlet. The cumulated entomological inoculation rate over the six months of study was only 0.018 infective bites in Bancoumana while it was up to 24.4 infective bites in the hamlet. About 55 cases of fever recorded at the health clinic between March and May; 38% had blood smear positive. All malarial fever cases were resident of Bancoumana (38.2% (21/55% of cases/total number of fever recorded March and May) and none from the Hamlet. In conclusion, the low level of malaria transmission in Bancoumana may be sustained by a high prevalence of infected mosquitoes in the Hamlet where numerous breeding sites were also found as result of drying river. A larval control strategy should target permanent water bodies during the dry season in those areas with seasonal malaria transmission.

1079

IN SEARCH OF ENVIRONMENTAL DETERMINANTS FOR MALARIA TRANSMISSIONS IN INDONESIA

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With a population of 242 million, Indonesia is the fourth most populous nation in the world. It also has the third highest malaria endemicity in Southeast Asia after Myanmar and India. Approximately 40% of its population lives in malarious regions. The distribution of malaria in Indonesia is highly heterogeneous. On Java and Bali, the two islands where about 70% of the population concentrates, malaria is hypoendemic. But on the Outer Islands, which include the rest of the archipelago, malaria

ranges from hypo- to hyperendemic. The Indonesian archipelago spans approximately 5,000 km. Within this wide geographic area, a number of dominant anopheles species are responsible for the major part of malaria transmissions. How the larval habitats of these species are affected by and respond to climatic, environmental, and human-induced changes are reflected in seasonal and longer-term variations in malaria transmissions. Understanding the ecology of these species also points to the way of using environmental management to reduce the propagation of malaria vectors. Passive and active case detection data from previous years for a number of Indonesian provinces are analyzed. Environmental parameters used in the study are extracted from surface observed and satellite measured datasets, including NASA's Tropical Rainfall Measurement Mission, Moderate Resolution Imaging Spectroradiometer, and Germany's Global Precipitation Climatology Center data. Using the neural network method, an artificial intelligence technique, we have characterized the relationship between malaria transmission and environmental parameters. The results may also be used for early warning malaria epidemics and outbreaks.

1080

EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF THE REEMERGING VIVAX MALARIA IN THE REPUBLIC OF KOREA

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Vivax malaria, which was prevalent in the Republic of Korea (= South Korea), disappeared rapidly since the 1970s, but re-emerged in 1993 near the demilitarized zone (DMZ), the border between South and North Korea. After the first reemergence, malaria prevalence increased exponentially, peaking in 2000, and then decreased; in total, 21,390 cases were reported between 1993 and 2005 in South Korea. The major infection source is, even at present, mosquitoes infected in North Korea and flying across the DMZ. Thus, this reemerging malaria is regarded as a peculiar type of border malaria; only mosquito vectors can come and go. During 1993-1997, the majority of cases (81.2%) were soldiers and veterans who worked in the northern parts of South Korea. However, during 2002-2005, soldier and veteran cases decreased (46.4%), and civilian cases (presumably local transmission) increased. The main transmission season is June to September each year; during this season vector mosquitoes *Anopheles sinensis*-complex, which includes *An. pullus* and *An. lesteri*, are very active. The reemerging malaria patients characteristically reveal combination of short (1-2 months) and long incubation periods (5-13 months) with predominance of the long type (2/3 of patients). Fever intervals are usually 48 hours, but frequently (20% of patients) atypical. Anemia is not commonly encountered, but thrombocytopenia is common. There are a few subclinical cases with apparent parasitemia. Various control measures have been operated, which must have greatly influenced the reduction of incidences.

1081

CHANGE IN MALARIAL PARASITEMIA PREVALENCE AND INDOOR RESIDUAL SPRAYING: EVIDENCE OF A DOSE RESPONSE RELATIONSHIP

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The Bioko Island Malaria Control Project (BIMCP) initiated a comprehensive malaria control intervention since February 2004, funded by a consortium led by Marathon Oil Company in partnership with the government of Equatorial Guinea (EG). The measures consist of indoor residual spraying (IRS) and case management including effective treatment based on combination therapy. Under the BIMCP all houses were sprayed once with pyrethroid insecticide in 2004; from January 2005 houses were sprayed with carbamates at 6 monthly intervals. Annual parasitemia household surveys were conducted in March 2004, 2005 and 2006 respectively

at 18 sentinel sites. A total of >10,000 children were tested in the three surveys. Household data included information on spraying, house construction, bednet use, illness histories and indicators of household wealth. Average prevalence of infection with *Plasmodium falciparum* at all sites combined reduced in all age groups 2 to <15 years from 45% at baseline to 31% in 2005 ($p<0.001$) and 26% in 2006 ($p<0.03$), with substantial between site variation. Reported site specific spray coverage of houses in the 2006 survey ranged from 58% to 87%. Odds of infection was significantly lower for children living in houses that had been sprayed (OR=0.67 relative to unsprayed houses, $p<0.001$), regardless of whether the child slept under a bednet or not. The odds of infection of an individual child decreased by a factor of 0.94 ($p=0.035$) for every one percent increase in spray coverage of the neighbourhood in which the child lived, independent of the spray status of her/his own home. Desire to have houses sprayed was uniformly high (mean 92%). Substantial overall reduction in prevalence of infection with *P. falciparum* in children can be achieved in equatorial settings provided a high level of spray coverage is maintained. Risk of infection is simultaneously related to the spray status of the child's home and to the spray-coverage achieved in the neighbourhood of the house.

1082

USING TREATMENT FAILURE TO SCREEN FOR MDR TB IS ASSOCIATED WITH RECURRENCE, DEATH AND TRANSMISSION

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In many resource-poor settings, TB drug susceptibility testing for all newly-diagnosed patients using traditional methods is not feasible. Instead, patients are given a trial of first-line drugs, and those who fail are then tested for MDR TB. This strategy seems to reduce both diagnostic and treatment costs because many MDR TB patients appear cured by first-line agents. No data exists, however, concerning the long term outcome of MDR TB patients "cured" by first-line drugs. 351 patients from a community hospital in Lima, Peru were enrolled after new diagnoses of TB disease. Patients were tested for resistance to rifampicin and isoniazid, followed throughout treatment, and interviewed a median of 60 months after treatment. This long term follow up established TB-related morbidity and mortality in both index cases and contacts. Cases of recurrence or contact TB were confirmed with health post records. Despite laboratory test results reporting 21 cases of MDR TB to patients and physicians, all 351 enrolled patients received a complete trial of first-line agents. Twelve of 21 patients (57%) with laboratory confirmed MDR TB converted to sputum smear negativity and were considered "cured" by first-line drugs alone. At long term follow up, however, patients cured of MDR TB were more likely to suffer recurrence (HR=18, 95%CI=7-45, $p<0.001$) and to die of TB (HR=7, 95%CI=1.4-38, $p=0.018$) than patients cured of non-MDR TB in Cox proportional hazard regressions accounting for differences in HIV prevalence and other risk factors for new TB infection. Among MDR TB patients, "cure" was not associated with a statistically significant reduction in long-term mortality ($p=0.3$). In addition, contacts of "cured" MDR TB patients suffered four times as many episodes of new TB as contacts of cured non-MDR TB patients during follow up (HR=3.8, 95%CI=2-9, $p=0.001$). There was similar contact TB incidence between MDR and non-MDR households prior to the study, implying that delayed MDR TB diagnosis may have caused increased transmission. In conclusion, the majority of MDR TB patients appeared cured by first line

agents, but usually this was a false cure, and MDR TB patients treated with first-line drugs were at high risk of TB relapse, death, and transmission. Using treatment failure to identify drug resistance underestimated MDR TB, and the resultant delays in optimal therapy were associated with mortality and MDR TB dissemination.

1083

HUMAN CELL-MEDIATED IMMUNITY AGAINST MYCOBACTERIUM TUBERCULOSIS ANTIGENS IS AUGMENTED BY TREATING INTESTINAL HELMINTHS

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Tuberculosis and intestinal helminth infections frequently co-exist and helminths cause immunosuppression, micronutrient deficiency and anergy. We hypothesized that treating intestinal helminths may augment antimycobacterial immunity. A double-blind, randomized, placebo-controlled trial in 140 healthy adults living in the Peruvian Amazon. Antimycobacterial immunity was assessed by measuring the size of cutaneous induration 48 hours after a 5 unit intradermal tuberculin skin test and by quantifying γ -interferon secretion following whole-blood stimulation with the specific *Mycobacterium tuberculosis* antigens ESAT-6 and CFP-10 (the Quantiferon in-the-tube assay). These *in vivo* and *in vitro* tests were performed at recruitment and 4 weeks after treatment with 3 daily doses of placebo or 400mg albendazole. Stools were examined by direct and concentrated quantitative microscopy. A stool examination at recruitment diagnosed intestinal helminths in 48% of 126 participants. 40% were infected with *Ascaris lumbricoides*, 12% *Trichuris trichuria*, 6.3% hookworms, and 3.2% *Strongyloides stercoralis*. Albendazole therapy caused helminth prevalence to fall to 6.9% (4/58) 2 weeks later ($P=0.003$) and to 15% (7/48) 4 weeks later ($P<0.001$). Eosinophil counts fell from median 271 cells/mm³ at recruitment to 201 cells/mm³ 4 weeks after albendazole therapy ($P=0.002$). At recruitment, 39% (52/135) of Quantiferon assays were positive and this did not change significantly with albendazole therapy. In contrast, 56% (78/139) of participants were tuberculin skin test positive at recruitment and albendazole therapy caused tuberculin skin tests to increase in size compared with placebo ($P=0.03$). In conclusion, treating intestinal helminths significantly augmented antimycobacterial immunity *in vivo* over a one month interval. Therefore, the interpretation of tuberculin skin test results may be complicated by antihelminthic treatment. Prevention or treatment of intestinal helminths warrants evaluation as a potential strategy for reducing tuberculosis susceptibility.

1084

BACTERIAL MENINGITIS AMONG 0- TO 15-YEAR OLD CHILDREN ADMITTED TO A PEDIATRIC REFERRAL CENTER IN BAMAKO, MALI

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Introduction of conjugate polysaccharide vaccines into infant immunization programs has resulted in dramatic declines in bacterial meningitis. However, global uptake of these vaccines has been slow, particularly in resource-poor countries of Africa. Barriers have included cost, few local demonstrations of impact, and lack of data on disease burden. To assist in quantifying the burden of meningitis in Mali, we performed hospital-based surveillance. Children 0-15 years old with fever $\geq 39^\circ\text{C}$ or suspected bacterial meningitis admitted to the major children's hospital in Bamako were eligible. A blood culture was collected from each child, and cerebrospinal fluid (CSF), obtained at the discretion of the treating physician, was also cultured. Microbiologically confirmed cases of meningitis were defined by the isolation of a pathogen from CSF. From June 2002 to May 2005, 5514 (90.6% of eligibles) were enrolled. Among the 3176 cases of clinically suspected meningitis, 2991 CSF samples were cultured (94%) and 558 yielded a pathogen (19%). Thus ~5% of hospital admissions had confirmed bacterial meningitis. The most common pathogens were *Haemophilus influenzae* type b (Hib; n = 247; 44%), *Streptococcus pneumoniae* (SP, n = 192; 34%) and *Neisseria meningitidis* (NM, n=42; 8%). 59% of SP belong to serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F, and 23F. Most NM were either type A (69%) or W135 (21%). Mortality was 19% among microbiologically confirmed cases. Adjusted independent risk factors for mortality were infection with SP (odds ratio [OR] 2.4; 95% CI 1.4-3.9), seizures at the time of admission (OR 2.3; 1.4-4.0), CSF WBC greater than 1,000/mm³ (OR 1.9; 1.1-3.2) and body temperature $\geq 40^\circ\text{C}$ (OR 1.9; 1.1-3.4). Receiving antibiotics in the week prior to admission was protective (OR 0.5; 95% CI 0.3-0.8). In conclusion, the incidence and mortality from bacterial meningitis in Mali is high, and most cases (~80%) are potentially vaccine-preventable. Ongoing introduction of routine Hib vaccine for infants should confer substantial benefits.

1085

BURDEN OF INVASIVE BACTERIAL INFECTIONS AMONG CHILDREN ADMITTED TO A PEDIATRIC REFERRAL CENTER IN BAMAKO, MALI - 2002 - 2006

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While much of the burden of childhood infection is borne by those in developing countries, most diagnoses are based on clinical findings and are not confirmed by laboratory studies. Due to limited resources, there is little data on the etiology and epidemiology of these infections. Such information is important for physicians who treat children in these settings as well as local decision makers who are seeking prevention methods. A clinical microbiology laboratory was established at the Hôpital Gabriel Touré. Children age 0-15 years with fever $\geq 39^\circ\text{C}$ or syndromes compatible with invasive bacterial disease (e.g. meningitis, pneumonia) and admitted to the hospital were eligible. Blood and relevant body fluid

(e.g. cerebrospinal fluid (CSF)) were cultured. Bacteria were identified by standard microbiologic techniques. From June 2002 to May 2005, 6087 of the 11671 children admitted to HGT were eligible and 5514 children (90.6%) were included. Most (4759; 86%) were from Bamako and 2839 (51.5%) were <1-year old. Pathogens were isolated in blood and/or other fluid from 1319 (24%) participants. The most common isolates among those children with fever without localizing signs were Gram negative bacilli (other than *Salmonella spp*) and non-typhoidal *Salmonella* (NTS). Those with suspected meningitis were more likely to have *Haemophilus influenzae* type b (Hib) and those with suspected pneumonia, *Streptococcus pneumoniae*. Among 0- to 11-month olds, 719 (25%) had a positive culture; Hib (n = 300) and *S. pneumoniae* (n = 201) were the most common isolates. Among 1- to 4-year olds, 360 (21%) had a positive culture; NTS (n = 110) and *S. pneumoniae* (n = 107) were most common. Among 5- to 15-year old children, 240 (25%) had pathogens, 66 were *S. pneumoniae* and 50 were NTS. In conclusion, the burden of bacterial invasive infections is high among children admitted to HGT. Vaccines against Hib and *S. pneumoniae* have the potential to greatly reduce this burden. Additional information regarding the source of gram negative bacillary and NTS infections is needed to design appropriate intervention strategies.

1086

AN EVALUATION OF A RAPID SERODIAGNOSTIC TEST FOR TYPHOID FEVER - AN GIANG, VIETNAM 2005-2006

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Typhoid fever causes over 21 million infections and 200,000 deaths worldwide each year. In Vietnam's Mekong Delta, the annual incidence is 198/100,000. Isolation of *Salmonella enterica* serotype Typhi from blood or bone marrow is the most reliable means of confirming acute typhoid fever. However, culture methods are costly, slow, and often unavailable in endemic areas. We assessed the validity of Tubex® TF, a rapid serodiagnostic assay for *S. Typhi* IgM, at the An Giang Province Hospital in the Mekong Delta. We compared Tubex® TF results to blood and bone marrow culture in hospitalized patients with fever for ≥ 3 days, temperature $\geq 38.5^\circ\text{C}$, and clinical suspicion of typhoid fever. We enrolled 123 patients from May 2005 to February 2006. The median age was 13 years (range 3-65); 43% were female. Symptoms included anorexia (91%), diarrhea (69%), nausea (68%), and abdominal pain (60%). Patients reported a median of 8 days (range 3-31) of fever before admission; the median admission temperature was 39°C (range 38.5-41.0). Blood cultures yielded *S. Typhi* in 45 (37%) patients; Tubex® TF was positive in all 45 and in an additional 62 in whom blood cultures were negative. Bone marrow cultures, performed in eight patients, yielded *S. Typhi* in five; Tubex® TF was positive in these five, including two patients whose blood cultures were negative. Tubex® TF was also positive in two of three patients with negative bone marrow cultures, one of whom had a positive blood culture. When compared to blood and bone marrow culture respectively, the sensitivity of Tubex® TF was 100% and 100%; specificity was 17% and 33%, positive predictive value was 42% and 71%, and negative predictive value was 100% and 100%. In conclusion, in an endemic area, Tubex® TF is highly sensitive but has poor specificity and positive predictive value compared to blood culture. Further comparison of Tubex® TF with bone marrow culture is warranted to more accurately assess the test characteristics before its routine clinical use can be recommended.

1087

CHARACTERIZATION OF LETHAL CASES OF LEPTOSPIROSIS WITH EMPHASIS OF WEIL'S SYNDROME AND SEVERE PULMONARY HEMORRHAGE SYNDROME, IN THE CITY OF SAO PAULO, BRAZIL

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Severe forms of leptospirosis are usually referred as Weil's syndrome - a triad of jaundice, hemorrhages and acute renal failure - and severe pulmonary hemorrhage syndrome (SPHS). SPHS is an emerging complication worldwide yet there is no clear explanation why its frequency and distribution is increasing. Recent reports from Nicaragua and Peru emphasize that leptospirosis may present as SPHS lacking jaundice or other typical manifestations of the Weil's syndrome. In Sao Paulo metropolitan area, Brazil, the incidence of severe leptospirosis ranged from 1.8 to 3.76 per 100,000 while fatality ranged from 11 to 18% in the last ten years. An active system of death notification including any combination of Weil's syndrome and SPHS, was started in 2003 in the city of Sao Paulo. AIM: to characterize the clinical features of lethal cases of leptospirosis in Sao Paulo with special focus on the emergence of SPHS. A cross-sectional study was performed in these death notifications from 2004 to 2006 of confirmed cases of leptospirosis. Lethal case was laboratorial confirmed by serology, culture or immunohistochemistry, or was based on epidemiological and clinicopathological grounds. Case fatality was 15% (42/285) in 2004, 11% (28/262) in 2005 and 11% (18/161) until May 2006. Case confirmation by a combination of serology, culture or immunohistochemistry was possible in 57 cases while 16 were identified based on clinicopathological findings and 15 on clinical and epidemiological grounds. In the period, the most common infecting serovars among cases and lethal cases, as predicted by microagglutination highest titer, were Copenhageni, Icterohaemorrhagiae, and Butembo. From 88 lethal cases, necropsies were performed in 31 cases detecting pulmonary hemorrhages in 24/31(78%) cases, being the cause of death of all the 24 patients, while Weil's syndrome was documented in 27/31(87%) by a combination of shock, renal failure and jaundice. SPHS and Weil's syndrome coexisted in 26/31 (84%) cases with fatal outcome. In conclusion, case fatality remains high in patients with severe leptospirosis and most deaths are consequence of SPHS. In contrast to other reports from other regions of the world, SPHS and Weil's triad usually coexist in patients with lethal outcomes from the city of Sao Paulo.

1088

CLINICAL AND LABORATORY COMPARISON OF HUMAN INFECTIONS WITH DENGUE, INFLUENZA, OR AVIAN INFLUENZA A (H5N1) VIRUSES IN INDONESIA

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Passive, hospital-based surveillance has been conducted in outpatients and inpatients from pediatric and internal medicine departments in up to 30 geographically balanced sites across the Indonesian archipelago

since 2004. Patients who met the criteria of influenza-like illness (fever >37.8°C and sore throat or cough) were enrolled and rapid influenza diagnostic tests were performed on site. Throat and nasal swabs shipped to Jakarta were screened for avian influenza A (H5N1) virus by real-time RT-PCR (rRT-PCR). Those found to be negative were cultured in MDCK cells and then analyzed by indirect immunofluorescence (IFA). H5N1 positive samples were shipped to the WHO Influenza Reference Laboratory at the Centers for Disease Control and Prevention for rRT-PCT, viral isolation and molecular sequencing. From January 2005 to March 2006, 853 influenza infections were confirmed including 27 influenza A (H1N1), 46 (H3N2), 307 influenza B and 28 H5N1 viruses. Retrospective analyses of the clinical and virologic characteristics were conducted on 56 patients diagnosed with influenza virus infection and 59 with dengue infection that were hospitalized at similar times and geographical locations. On average, patients with human influenza virus infections were hospitalized on day 3.4 of illness, H5N1 cases on day 4.9 and dengue infections on day 4. The clinical manifestations of all influenza viruses during the first three days were similar (fever, cough, and sore throat). However, dyspnea and bronchopneumonia were found predominantly in patients with H5N1 three to ten days after disease onset (mean: 4.8 days). The mean leukocyte, platelet and lymphocyte counts of H5N1 patients over the first five days of illness were significantly lower (p 0.05) than values among other influenza A or B viruses. These findings demonstrate that among a pool of patients seeking medical care in Indonesia during the initial forty-eight hours after onset of illness, H5N1 infections were often indistinguishable from human influenza or dengue infections. This may contribute to a delay in diagnosis and treatment of patients with H5N1.

1089

TRANS-SPLICING OF THE DEN2-NGC GENOME AT A HIGHLY CONSERVED SITE BY A GROUP I INTRON RIBOZYME

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The trans-splicing variant of the *Tetrahymena thermophila* group I intron catalytic RNA, or ribozyme, is a powerful tool for post-transcriptional RNA modification. It can be directed to act upon a specific accessible uracil on its RNA substrate through controllable base-pair interactions. In the trans-splicing reaction, a target RNA is cleaved at a precise point and a new exon from a separate RNA molecule is then covalently joined to the upstream cleavage product to create a new mRNA ready for translation. The nature of the ribozyme and the predictability with which it can be directed makes it a powerful tool for modifying RNA in nearly any cell type without the need for genome-altering gene therapy techniques or dependence on native protein cofactors. We have successfully targeted two different uracil bases on the positive sense strand within the highly conserved region common to all serotypes of dengue with the group I intron. Our ribozymes have demonstrated the ability to specifically cleave these bases and covalently trans-splice a new RNA sequence downstream of the targeted site, allowing for *de novo* gene expression triggered by dengue viral infection of the cell. This approach provides a two-tiered response of dengue genome cleavage and new protein expression to the presence of the dengue genome. By altering the configuration of the RNA interactions during the trans-splicing reaction and measuring activity by real-time RT-PCR, we have been able to improve the efficiency with which the intron catalyzes the reaction at the two target sites without altering the functional sequence within the intron itself. We envision this novel approach of infection-triggered *de novo* gene expression to be of great potential in combating the spread of dengue virus at the insect level.

1090

ROLE OF STRESS RESPONSE MOLECULES IN DENGUE VIRUS INFECTION IN MOSQUITO AND HUMAN CELLS

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The first step in dengue virus infection is the interaction between the envelope (E) protein, with the cellular receptor on the cell surface. Several molecules have been described as part of dengue virus receptor complex in different cell lines, suggesting that the interaction between dengue virus and its receptor(s) is a complex, multi-step process or dengue virus may require a receptor complex formed by a group of molecules present in different susceptible cells. Our group has identified the participation of two heat shock proteins (HSPs), HSP90 and HSP70 as part of dengue virus receptor complex in human cells. They were found associated with membrane microdomains (lipid rafts), whose integrity is important for dengue virus entry. Since during dengue infection a systemic inflammatory stress response is evident, we specifically aimed to determine the role of stress mediators in dengue virus infection in human and mosquito cell lines. We found that during stress conditions, viral entry in mammalian cells and binding in mosquito cells are increased. Additionally, reactivity of antibodies directed against HSP90 to the gp45, the glycoprotein involved in dengue infection in C6/36 cells, and its relocation during stress suggests that gp45 is a HSP-like molecule. On the other hand, molecules involved in innate immune response such as the toll like receptor 4 (TLR4), are relocated to lipid rafts in response to dengue virus interaction. It is possible that different molecules play important roles in Dengue virus entry and/or cell signaling, priming events that could trigger the cellular response to dengue virus infection.

(ACMCIP Abstract)

1091

ROLE OF DC-SIGN AND FCGR2 IN ANTIBODY DEPENDENT ENHANCEMENT OF DENGUE INFECTION

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We established a flow-based assay system to study antibody-dependent enhancement (ADE) of Dengue virus infection. We have applied this technique to cell lines expressing varied levels of DC-SIGN and different subsets of receptors from the FcγII family. There is a negative correlation between the level of DC-SIGN, and a positive correlation with the expression of particular Fc receptors, to the ability of cells to undergo ADE. We have expanded these studies to evaluate ADE in primary human dendritic cells and monocytes, testing several donors with a variety of dengue immune sera. Using blocking antibodies and directed knockdown via siRNA, we can selectively inhibit expression of the Fc receptors and DC-SIGN, either individually or in combination. Early evidence points to opposing roles for the isoforms of FcγRII (A and B) in ADE. Dengue virus requires the presence of FcγRIIA on K562 cells in order for ADE to occur. Cells with high levels of DC-SIGN do not undergo ADE, but in cells with low DC-SIGN, FcγRIIB appears to inhibit ADE. We will report our current data and progress thus far towards understanding the mechanism of ADE.

1092

DEVELOPING A MOUSE MODEL OF SECONDARY DENGUE VIRUS INFECTION

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Primary infections with dengue viruses (DENV) are typically asymptomatic or result in self-limited dengue fever (DF). Upon second infection with a different DENV serotype, however, patients are much more likely to develop life-threatening Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS). Increased disease severity in secondary DENV infections is believed to result at least in part from the phenomenon of antibody-dependent enhancement (ADE), in which anti-DENV antibodies are present at levels high enough to bind to the virus, but too low to efficiently neutralize it. Uptake of virus-antibody complexes by Fcγ receptor-bearing immune cells could then result in increased infection of these cells, which may lead to increased viremia and contribute to pathogenic immune dysfunction. Many aspects of the ADE phenomenon remain poorly understood, however, due to the lack of an appropriate small animal model. To explore the mechanism of secondary DENV infection *in vivo*, two approaches were used. In the first model, interferon α/β x γ receptor-deficient (AG129) mice were not infected or infected with the DENV1 Mochizuki strain at 5-6 weeks of age, then administered a secondary infection with DENV2 strain PL046 5 or 15 weeks later. At both time intervals, primary infection with DENV1 appeared to be protective in that reduced viral load was observed upon DENV2 infection of DENV1-immune mice compared to naïve mice. Other combinations of virus strains and longer intervals between sequential infections are currently being tested. In the second model, anti-DENV1, 3, or 4 immune serum was generated and tested *in vitro* for binding, neutralizing and enhancing activity against the homologous virus as well as DENV2. Antisera with enhancing activity *in vitro* will be passively transferred into AG129 mice prior to infection with DENV2. Viral load will be measured in various tissues to determine if the antisera enhanced infection. These approaches should shed light on the mechanism of ADE and the pathogenesis of secondary DENV infection *in vivo*.

1093

DENGUE VIRUS TARGETS MACROPHAGES AND DENDRITIC CELLS IN A MOUSE MODEL OF INFECTION

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Dengue fever is a mosquito-borne viral illness caused by one of four dengue viruses (DENV1-4), resulting in ~100 million infections each year in tropical and sub-tropical regions. A percentage of symptomatic infections proceed to a more severe, life-threatening form characterized by increased vascular permeability, designated dengue hemorrhagic fever/dengue shock syndrome. The molecular basis of DENV pathogenesis is not well understood, however, due in part to lack of a representative animal model in which to test hypotheses generated by clinical and epidemiological observations. The cellular tropism of DENV in humans has not been fully defined, although viral antigen has been detected in dendritic cells in skin biopsies and in monocytes from peripheral white blood cells (WBCs). We find that the initial cellular tropism of DENV in mice and humans is similar, even though the phenotypic endpoint of DENV infection in mice is usually encephalitis. 129/Pas mice lacking IFN-α/β and -γ receptors (AG129) were infected with DENV via a subcutaneous (sc) route to approximate a mosquito bite. Two DENV2 strains were used: a Taiwanese clinical isolate (PL046) as well as a mouse-passaged virus recently derived in our laboratory from PL046 (D2S10) that results in increased vascular permeability in mice. During the first week after sc infection with either DENV2 strain, virus is present primarily in the lymph nodes, spleen, bone

marrow, and circulating WBCs as measured by plaque assay. Of interest, the D2S10 strain displays an increased titer of virus in the bone marrow and WBCs at early timepoints post-infection. Flow cytometric analysis demonstrates that CD11b⁺ macrophages and CD11c⁺ dendritic cells are targeted by DENV2 *in vivo*. We have also detected infectious DENV and viral RNA by plaque assay and strand-specific RT-PCR, respectively, in both CD11c⁺ and F4/80⁺ spleen cells that were separated using antibody-labeled magnetic beads. In addition, bone marrow-derived macrophages from AG129 mice are susceptible to DENV2 infection *in vitro*, and increased titers of virus are recovered when sub-neutralizing levels of anti-DENV2 antibodies are present. Our data indicates that the initial cellular targets of DENV in mice and humans are the same -- primarily macrophages and dendritic cells, thereby allowing investigation of the tropism and pathogenesis of the virus at the cellular level in primary and secondary DENV infections in an *in vivo* mouse model.

1094

PHENOTYPING OF PERIPHERAL BLOOD MONONUCLEAR CELLS INFECTED BY DENGUE VIRUS IN PEDIATRIC CASES

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Although dengue virus (DENV) and DENV antigen have been detected in peripheral blood mononuclear cells (PBMC) *in vivo*, controversy remains regarding the major cell type infected and the frequency of infected cells. To identify which sub-populations of PBMCs are infected by DENV in humans with symptomatic dengue illness, flow cytometric analysis was performed on samples from pediatric cases of dengue in Nicaragua. A prospective study was conducted in the National Pediatric Reference Hospital in Managua from August 2005 to January 2006. Blood samples were obtained daily from suspected dengue cases recruited ≤ 4 days since onset of symptoms, and PBMCs were prepared using CPT tubes (ficoll/hypaque separation). Dengue virus antigens were identified using antibodies directed to the prM protein (monoclonal antibody 2H2). PBMCs were phenotyped using 2 cocktails of antibodies, one containing labeled monoclonal antibodies directed to CCR7, CD209, CD16, CD83, and CD86, and the other consisting of labeled antibodies targeted to CD8, CD4, CD32, CD14, and CD11c. Positive samples were then further analyzed by staining with CD20, as well as intracellular cytokine staining. The presence of DENV was also tested by RT-PCR and virus isolation in serum samples obtained from the patient at the time of PBMC collection. In preliminary results, PBMCs from 18/20 samples were positive for DEN antigen by 2H2 staining. All cells positive for DENV antigen expressed CD86 (the co-stimulatory molecule B7-2) and CD32 (Fc γ RII receptor). Increased CD209, CCR7, and CD16 (Fc γ RIII) expression appeared to correlate with higher levels of DENV antigen. These initial studies indicate that the sub-population of PBMCs infected by DENV is predominantly monocytes/dendritic cells, perhaps activated in the presence of immune complexes. In addition, DC-SIGN may not be the major DENV receptor of PBMCs *in vivo*, since the percentage of cells positive for DC-SIGN was much lower than the total percentage of infected cells. PBMCs from additional patients as well as serial daily samples are currently being evaluated using the same methodology.

1095

DIFFERENT SUBSETS OF PRIMARY HUMAN CELLS HAVE DIVERGENT SUSCEPTIBILITY TO DENGUE VIRUS (DV) INFECTION AND CAPACITY TO MEDIATE ANTIBODY-DEPENDENT ENHANCEMENT (ADE)

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ADE is postulated to be a major mechanism for causing dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). What specific cells mediate ADE, however, have been controversial. Many cell types have been implicated in ADE, including monocytes/macrophages, dendritic cells, Langerhans cells, endothelial cells, T cells, B cells and hepatocytes. The relative DV permissiveness among these cells and their intrinsic ability to mediate ADE are not yet known. To address these issues, we exposed freshly isolated human peripheral blood mononuclear cells to a virulent strain of DV2 (16681) in the presence or absence of pooled dengue-immune human sera (PHS), and assessed infection using novel monoclonal antibodies (MAb) against NS1 or E proteins by 8-10 color flow cytometric analysis. In parallel, we measured DV infection by conventional Western blot and reverse-transcriptase PCR techniques. In 10 healthy donors, we found that monocytes were the principal cells for DV infection without PHS (3.85 \pm 3.54% positive for anti-E MAb staining), and have the greatest capacity to mediate ADE (9.99 \pm 6.29%) in the presence of highly diluted PHS. In contrast, no T or B cells were infected with or without the addition of PHS (<0.02%). Interestingly, some cells lacking T, B or monocyte markers were also infected (0.36 \pm 0.15%) in the presence of highly diluted PHS. Furthermore, subsets of monocyte are not equally susceptible to DV infection, nor do they have the same capacity to mediate ADE. These results provide more insight into the pathogenesis of DHF and DSS.

1096

FORMULATION DEVELOPMENT OF A CHIMERIC MALARIA VACCINE CANDIDATE (PFCP2.9) WITH MONTANIDE ISA 720 FOR CLINICAL EVALUATION

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PfCP2.9 is a recombinant fusion protein consisting of AMA1 (domain III) and MSP1-19 that is undergoing development and evaluation as a vaccine candidate against *Plasmodium falciparum* malaria. Both AMA1 and MSP1 are located on the merozoite surface and are believed to play a role in the invasion of red blood cells. PfCP2.9 is secreted at high levels from GS115 *Pichia pastoris* with a yield of 1g/L at 30-L scale fermentation. More than 30% of the protein is recovered from a purification process involving phenyl hydrophobic interaction, ion-exchange, and gel filtration chromatography, with >98% purity. To date, the PfCP2.9 bulk remains stable at -70°C for at least one year. PfCP2.9 is undergoing Phase 1 clinical evaluation with Montanide ISA720. PfCP2.9 is emulsified with ISA 720 by homogenization to form a white water-in-oil emulsion with a mean particle size of approximately 1 μ m. The stability of the PfCP2.9/ISA720 formulation stored at 4°C is assayed by particle size distribution and SDS-PAGE of extracted antigen. The antigen remains unchanged for up to three months; thereafter, aggregation and degradation of PfCP2.9 is seen by SDS-PAGE of extracted antigen and this increases over time. After nine months, only 50-60% of the extracted antigen is full length. The addition of glycine to the PfCP2.9/ISA 720 formulation was shown to prevent modification of the antigen and to date, no modification of PfCP2.9 can be seen for up to nine months with storage at 4°C. We propose that the addition of glycine can stabilize the PfCP2.9/ISA 720 formulation and

should be included in all future clinical formulations with this antigen and adjuvant.

1097

A PHASE 1 DOUBLE-BLIND, RANDOMIZED, CONTROLLED STUDY OF AMA-1/MSP-1 RECOMBINANT MALARIA VACCINE (PFCP-2.9/MONTANIDE ISA 720): A BLOOD STAGE VACCINE FOR *PLASMODIUM FALCIPARUM* MALARIA

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A vaccine that can reduce both morbidity and mortality from *Plasmodium falciparum* infection is the goal of malaria vaccine development. Wanxing Bio-Pharmaceuticals reports on a Phase 1 double-blind randomized controlled trial of PfCP-2.9, a malaria vaccine candidate adjuvanted with Montanide ISA 720. PfCP-2.9 is a recombinant chimeric protein expressed from *Pichia pastoris*, consisting of Apical Membrane Antigen-1 (domain III) and the 19 kDa portion of Merozoite Surface Protein-1 from the 3D7 and K1 *P. falciparum* lines, respectively. The primary objective of the trial at the Shanghai Changhai Hospital in China is to assess the safety and reactogenicity of PfCP-2.9/ISA 720 in healthy, adult volunteers. The secondary objective is to assess the vaccine's immunogenicity by evaluating and comparing antigen-specific antibody responses (anti-PfCP-2.9 ELISA) after each vaccination.

This trial, approved by three ethics committees, is being conducted in collaboration with the PATH Malaria Vaccine Initiative and the World Health Organization. Seventy volunteers who met the protocol-defined inclusion and exclusion criteria were randomly assigned to one of three dose cohorts (5 µg, 20 µg and 50 µg). Volunteers in the 5 and 20 µg cohorts were randomized a second time to one of two vaccination schedules (A: 0, 60, 180 days or B: 0, 90, 180 days). Volunteers in the 50 µg cohort were vaccinated according to schedule B. Within each of the five groups, 14 volunteers were randomized to receive either vaccine or control (10 volunteers received vaccine and four volunteers received Montanide ISA720 adjuvant alone). For both schedules A and B, the volunteers had 20 follow-up visits during their 240 days to check local and systemic adverse events (AE). Blood samples were taken for hematological and biochemical tests to assess safety, and for immunological analyses (anti-PfCP-2.9 ELISA, growth inhibition assay and immunofluorescence assay).

1098

PHASE 1 SAFETY AND IMMUNOGENICITY TRIAL OF MSP1₄₂-FVO/ALHYDROGEL AND MSP1₄₂-3D7/ALHYDROGEL BLOOD-STAGE MALARIA VACCINES IN US ADULTS

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A Phase 1 clinical trial has been conducted in healthy US adults to compare the safety and immunogenicity of 2 recombinant protein vaccines based on *Plasmodium falciparum* MSP1₄₂-FVO and MSP1₄₂-3D7, the 2 dimorphic forms of the blood-stage antigen. Volunteers were given 3 vaccinations of either 5, 20 or 80 µg protein adsorbed to Alhydrogel at 0, 1 and 6 months. All vaccinations were well tolerated. Antibody levels to homologous and heterologous MSP1₄₂, MSP1₁₉ and

MSP1₃₃ were measured by ELISA pre-vaccination, collected 2 weeks after each vaccination and during the 6 month follow-up period. In a proportion of volunteers, elevated MSP1₄₂ specific antibody levels were present 2 weeks following the second vaccination and were boosted by subsequent vaccination. Following the third vaccination, MSP1₄₂ antibody responses varied from undetectable to a titer of over 3,000 (reciprocal dilution at an OD of 1). The elevated MSP1₄₂ specific antibody levels diminished with time prior to the six month boost but were still detectable 6 months following the final vaccination. No correlation between MSP1₄₂ antibody levels and vaccine dose was found. MSP1₁₉ specific antibodies were detected and correlated with MSP1₄₂ antibodies. Little MSP1₄₂ strain specificity in the anti-MSP1₄₂ and anti-MSP1₁₉ responses was detected by ELISA and immunofluorescence of infected red blood cells. This is in contrast to the MSP1₄₂ specific T cell responses described in a companion paper. Antibodies to the homologous MSP1₃₃ protein were observed following three vaccinations in some volunteers. No parasite growth inhibition was detected in an *in vitro* assay using purified antibody from the volunteers. The results of this clinical trial were not predicted by preclinical animal studies that showed MSP1₄₂-FVO was more immunogenic than MSP1₄₂-3D7 and a part of the antibody responses were strain specific. Due to the modest immune responses of MSP1₄₂/Alhydrogel vaccines, further development is underway to evaluate potentiation of the immune response by the addition of CPG 7909.

1099

T CELL RESPONSES IN VOLUNTEERS VACCINATED WITH BLOOD-STAGE MALARIAL ANTIGENS MSP-1 AND AMA-1

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The malaria merozoite proteins AMA-1 and MSP1 have been investigated as potential candidates for a malaria vaccine because of their pivotal role in the invasion of *Plasmodium falciparum* parasites into red blood cells. Since our knowledge of T cell responses in humans to these antigens is limited, we evaluated the nature and specificity of T cell responses in healthy American volunteers vaccinated with two leading blood-stage vaccine candidate antigens - MSP1₄₂ and AMA1-C1. Both proteins were formulated on Alhydrogel in two different Phase I Clinical trials. Antigen-specific T cell responses were assessed by ELISPOT assays, intracellular staining for IL-5 and IFN- γ , and ELISA determinations for several cytokines secreted upon re-stimulation with specific vaccine antigen. In MSP1 vaccinated volunteers, specific T cell immune responses were modest for Th1 cytokines such as IFN- γ or IL-2; however, Th2 cytokines like IL-5 and IL-13 showed increases after the third vaccination. Similar findings were observed in the volunteers vaccinated with AMA1-C1. These results indicate that the responses to alum adjuvanted vaccines in humans predominantly followed a Th2-type pattern. Two groups of MSP1₄₂ vaccinated volunteers were evaluated for allele specificity after one group was vaccinated with the MSP1₄₂ FVO allele and another was vaccinated with MSP1₄₂ 3D7. Interestingly, when responses to homologous and heterologous antigen were compared in each group, IL-5 responses were significantly higher for the homologous antigen, both in supernatants, and in the number of IL-5 secreting cells. In contrast, antibodies from the same individuals showed nearly complete cross-reactivity. This suggests that the T cell responses are more allele-specific than the B cell responses. The same malarial antigens formulated in Alhydrogel with CPG 7909 were also tested in Phase 1 Clinical Trials, and the specific cellular responses were evaluated. Results will be presented on the implications for the choice of antigens and adjuvants for candidate malaria vaccines.

(ACMCIP Abstract)

1100

RANDOMIZED, CONTROLLED, PHASE 1 STUDY OF AMA1-C1/ALHYDROGEL® VACCINE FOR PLASMODIUM FALCIPARUM MALARIA IN CHILDREN IN DONÉGUÉBOUGOU, MALI

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Apical membrane antigen-1 (AMA1) - one of the leading malaria vaccine candidates - is a surface protein expressed during the asexual blood stage of *Plasmodium falciparum* that has been implicated in parasite invasion of erythrocytes. AMA1-C1/Alhydrogel® consists of an equal mixture of recombinant AMA1 from the FVO and 3D7 clones of *P. falciparum* that is adsorbed onto Alhydrogel®. A Phase 1 study in semi-immune adults in Mali has shown that the vaccine was safe and immunogenic particularly in those who received the 80 µg dose. A Phase 1 study in healthy children aged 2-3 years was started in March 2006 in Donéguébougou, Mali. Thirty-six children were enrolled into one of the two-dose-groups (n=18/ group) and randomized 2:1 to receive either AMA1-C1/Alhydrogel® or *Haemophilus influenzae* type b Hiberix® vaccine. The 1st and 2nd dose-groups were vaccinated successively at 3-week intervals, and received 20 and 80 µg of AMA1-C1, respectively. Vaccinations were administered on days 0 and 28 and participants were examined on days 1, 2, 3, 7, and 14 after each immunization and then about every two months for 1 year. Of 36 volunteers enrolled, 33 received both vaccinations. As of study day 42 of the second group, there have been 16 local, systemic and laboratory abnormalities related to the vaccination. All have been mild. Seven participants had elevated alanine-aminotransferase (ALT) due to acute Hepatitis A infection; three of these children were hospitalized briefly. No vaccine-related serious or grade 3 adverse events have been observed. There was no increase in local, systemic or laboratory abnormalities with respect to increasing dose of vaccine or increasing number of immunizations. The blinded data so far suggest that AMA1-C1/Alhydrogel® is well tolerated in children. Based on these safety results, a Phase 2 study in children is being undertaken. Additional safety results up to Day 154 will be presented.

1101

HUMAN MALARIA-SPECIFIC IFN- γ T CELL RESPONSES INDUCED BY VIRUS LIKE PARTICLES, COMPRISED OF HEPATITIS B VIRUS CORE ANTIGEN AND P. FALCIPARUM CIRCUMSPOROZOITE PROTEIN T AND B CELL EPITOPES (ICC-1132), ADJUVANTED WITH ALUM AS COMPARED TO ISA 720

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Virus like particles (VLP) can provide a promising malaria vaccine platform as shown by recent clinical trials of RTS,S, a VLP comprised of Hepatitis B surface antigen and truncated *Plasmodium falciparum* CS protein. RTS,S vaccine efficacy required a complex adjuvant formulation containing an oil-in-water emulsion, QS21 and monophosphoryl lipid A. We have

investigated a VLP vaccine comprised of recombinant Hepatitis B core antigen containing minimal T and B cell epitopes of *P. falciparum* CS protein (ICC-1132). In previously reported Phase I trials, ICC-1132 elicited positive anti-sporozoite antibody and CS-specific T cells in the majority of vaccinees. A critical role of CS-specific IFN- γ producing CD4+ T cells in resistance to *P. falciparum* sporozoites has been shown in recent studies in vaccinated and naturally-infected humans. We therefore examined the kinetics and fine specificity of human IFN- γ producing CD4+ T cells induced by multiple doses of ICC-1132/alum as compared to responses obtained following a single dose of ICC-1132 formulated in the more potent water-in-oil adjuvant, Montanide ISA 720. The majority of volunteers primed and boosted with ICC-1132/alum developed CS-specific T cells detectable by cultured IFN- γ ELISPOT and by measurement of IFN- γ in supernatants using the Cytokine Bead Assay (BD Biosciences). A single dose of vaccine formulated in ISA 720 also elicited malaria-specific IFN- γ producing T cells detectable by these assays. The majority of cells were specific for the T* universal T cell epitope, with minimal responses to the CS repeats. In both adjuvant formulations, a Th1/Th0 cytokine profile predominated, with IFN, IL-2 and TNF α , but minimal IL4/5, detectable in the majority of culture supernatants. These findings suggest that the ICC-1132 VLP vaccine formulated in readily available adjuvants can induce CD4+ T cells that can target the parasite at multiple stages by secreting helper factors for production of sporozoite neutralizing antibody and by IFN- γ mediated inhibition of intracellular hepatic stages.

(ACMCI Abstract)

1102

INVASION INHIBITION OF P. VIVAX BY ANTI-DUFFY BINDING PROTEIN ANTIBODIES

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Plasmodium vivax (Pv) is the second most prevalent malaria species infecting humans accounting for half of all malaria cases in Latin America and Asia, and 70 million cases annually. Numerous studies have shown that Pv merozoites interact with the Duffy blood group antigen in order to complete the red blood cell (RBC) invasion process. Additional studies have identified the parasite binding ligand, Pv Duffy Binding Protein (PvDBP) and amino acids that interact directly with the human Duffy blood group antigen. Interference with, or disruption of this ligand:receptor interaction may limit Pv invasion of target reticulocytes, making the PvDBP an attractive vaccine candidate. To examine the influence of antibodies on Pv viability we tested two antibody solutions specific for the PvDBP Sal I allele for their ability to inhibit Pv invasion in short-term *in vitro* cultures. Parasites for these cultures were obtained from Pv-infected individuals from the Mae Sot region of Thailand. Following depletion of leukocytes (CF11 cellulose column), parasite cultures were established in cultures in McCoys 5A + 25% Human AB serum using autologous RBC. Antibodies included a rabbit polyclonal antiserum raised against recombinant PvDBP, and an antigen-specific, affinity purified, pooled antiserum from 14 Pv-infected Papua New Guineans. Results from our studies found that addition of rabbit anti-PvDBP (1:100) to short-term Pv cultures reduced the number of invasion events by an average of 66% (p=0.07). Affinity-purified antiserum from Pv-infected Papua New Guineans (100 µg/mL) reduced Pv invasion by 43% (p<0.01). These results show for the first time that both rabbit and human antibodies directed against the PvDBP Sal I allele reduce RBC invasion efficiency by Pv.

(ACMCI Abstract)

1103

HUMAN HOOKWORM VACCINE TRIAL: MODELING TRIAL EFFICACY AND HEALTH IMPACT

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To help develop a new tool for the control of human hookworm, the Human Hookworm Vaccine Initiative (HHVI) has identified and produced several vaccine candidates, the most promising being the Na-ASP-2. The first-in-man study has been completed in healthy US volunteers and a phase 2 trial in an endemic area of Brazil is currently in preparation. The vaccine is expected to be non-sterilizing, but still capable of slowing down the post-treatment re-infection process, after chemotherapy. Little is known however about the efficacy and waning time both at the individual and population level, and no reliable animal model for this infection exists. To help explore the possible trial results and potential global health impact, we are developing a mathematical framework to quantify the consequences of vaccination and to define the desired characteristics of an efficacious. As an initial step, we investigate putative ecological and immunogenetic factors underlying observed patterns in hookworm infection, through a tailored individual-based computer simulation. This allows for a stratified analysis of the population and for the exploration of different scenarios, dependent on biological determinants (worm fertility and death rate, human genetic predisposition and age, environmental factors) as well as on behavioral factors (individual habits and social mixing). As a function of level of coverage, efficacy and waning-time, we estimate the direct benefit provided by the vaccine. A special emphasis is put on the role played by children and adult sub-populations in the hookworm transmission dynamics and on the determinants and epidemiological consequences of spatial heterogeneity of infection. A sensitivity and sample-size dependency analysis provide focus for the design and the analysis of the vaccine trial. We find that both immunogenic and behavioral heterogeneities are likely to play crucial, although different, roles in the dynamics of hookworm infection and will both need to be taken into account when choosing size and (possibly) composition of the vaccine trial population sample. In addition, we identify the vaccine efficacy and waning required for the vaccine to have a substantive impact on mean worm relative to proving chemotherapy alone.

1104

PARASITE RISK FACTORS FOR UNDERWEIGHT AND WASTING IN PRESCHOOL-AGE CHILDREN IN BELEN, PERU USING THE NEW WHO INTERNATIONAL GROWTH STANDARDS

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Background: Recently WHO released a new set of international growth standards with which more accurate estimates of malnutrition could be calculated. This information is particularly valuable in areas of extreme poverty as it can ensure that risk factors for malnutrition, including parasite infections, are properly identified and appropriate interventions targeted. The objective of this study was to determine accurate estimates of malnutrition indicators and associated risk factors in a population of preschool-age children in Belen, Peru, a community of extreme poverty. A household survey was conducted in Belen from Oct 2005 to Jan 2006. Demographic information and various measurements (anthropometry, blood and stool samples) were collected from one child under five from each household. Anthropometric calculations were made using the WHO Anthro 2005 software. Wasting and underweight were defined as > 2 SD

from the WHO reference population for height-for-weight and weight-for-height, respectively. Multivariable logistic regression was used to determine the independent risk factors for wasting and underweight. A total of 252 children with complete anthropometric measurements, and blood and stool samples were included in the analysis. Mean age was 27.5 months (± 16.2). Forty-nine percent were female. The prevalence of wasting and underweight was 26.6% and 28.6%, respectively. Risk factors for underweight included mother's education level (secondary incomplete vs. secondary complete) (OR=3.04; 95%CI: 1.47, 6.29), moderate-high intensity of *Trichuris* infection (OR=4.78; 95%CI: 2.08, 11.02), and age of child (OR5-month=1.14; 95%CI: 1.04, 1.26). The above risk factors and one additional risk factor (hookworm infection (OR=6.41; 95%CI: 1.08, 38.5)) were also associated with wasting. The results indicate the presence of parasite risk factors for malnutrition in children under five in this community of extreme poverty. Cost-effective strategies for deworming and nutrition interventions that target mothers and preschool children are urgently required.

1105

RECENTLY IDENTIFIED BACILLUS SPECIES PRODUCER OF POTENT NEMATOCIDAL COMPOUNDS

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Nematode diseases including elephantiasis and onchocerciasis still persist, and affect millions particularly in developing countries. Present drugs including Mebendazole and Diethylcarbamazine are somewhat effective. We continue to investigate the effects of the purified component of an extract obtained from *Bacillus mojavensis* strain 14135 nov. The fractionated protein was analyzed utilizing GC/MS. Varying concentration of the purified fraction was added to culture-plates containing 1st through 4th stage larvae of *Caenorhabditis elegans* (*C. elegans*). This study examines the effects of these various concentrations on different larvae of *C. elegans*. 1st to 4th stage larvae of *C. elegans* were cultured in Luria broth in 24 well tissue-culture plates. The medium was supplemented with 5mg/ml cholesterol and OP50 strain *E. coli* previously cultured in 2xYeast Tryptone broth. We subsequently treated test wells with varying concentration of the fraction from .05 μ g/ml-10.0 μ g/ml. The fractions contained a number of recently identified lactones including Pyrrolo [1, 2-a] piperazine-3, 6-dione and Pyrrolo [1, 2-a] pyrazine-1, 4-dione. We checked for the lack of motility in the different larval stage over a 72-hour period, and examined worms for structural damage. We recorded the absence or presence of motility which begun at approx. 15 minutes post-treatment. Muscular activity appeared to be totally absent after the 72 hours. Later larval stages appeared to be affected more readily. However, all larval stages subsequently succumbed to the compounds within the extract. Conclusions: It appears that the compound(s) Pyrrolo [1, 2-a] piperazine-3, 6-dione and Pyrrolo [1, 2-a] pyrazine-1, 4-dione which were identified by GC/MS fractionation are exceedingly toxic to all larval stages. Further studies to understand the activity of the identified compound(s) will be conducted. However, it appears that these compound(s) could be of tremendous importance in the future regarding the fight against several nematode infections and other parasitic organisms.

(ACMCI Abstract)

1106

THE INFLUENCE OF HELMINTHS ON IMMUNE RESPONSES TO HIVZilungile L. Kwitshana¹, Gerhard Walz², John E. Fincham¹¹South African Medical Research Council, Durban, South Africa,²Stellenbosch University, South Africa, Cape Town, South Africa

Differences in the AIDS epidemic have been documented to exist between developing countries and the Western world. Several factors responsible for this variation have been suggested. Background immune activation by infectious agents common among poor communities of developing countries has been widely implicated. Helminths have been shown to play a significant role in inducing chronic immune activation in these populations. Increased susceptibility to HIV infection and faster progression to AIDS are postulated to result from pre-existent immune dysregulation under these conditions. The aim of this study was to define the immune profile of HIV-1 seropositive and seronegative individuals with or without helminth co-infection in a resource-poor setting. The hypothesis tested was that helminths alter immune responses to HIV infection by a shift from Th1 to Th0/Th2 phenotype and enhance susceptibility to HIV/AIDS. Baseline and follow-up blood and fecal samples obtained from 128 HIV-1 seropositive and 45 HIV seronegative individuals were collected at intervals after a 15-month period of deworming. Evidence for helminth exposure was measured by fecal egg excretion and elevated *Ascaris* specific IgE. Lymphocyte phenotypes and viral loads were compared between groups infected or uninfected with helminths and HIV-1. Currently, activation status, lymphocyte proliferative responses and cytokine production are investigated between subgroups. Fifty-three of 128 HIV-1 positive and 18 of 45 negative subjects had evidence of worm infection. Lower median CD4 and higher CD8 counts were obtained from HIV-1 positives compared to negatives. In the former, higher median viral loads were associated with helminth infection. However, higher CD4 counts were found in helminth-infected groups with or without HIV. In conclusion, these preliminary results are equivocal thus further analysis of the complete immune profile among the groups will elucidate whether helminth exposure alters the response to HIV infection.

1107

CYTOKINE RESPONSES TO STRONGYLOIDES STERCORALIS INFECTIVE-STAGE LARVAL ANTIGEN IN STRONGYLOIDIASIS PATIENTS WITH HTLV-1 CO-INFECTIONMartin Montes¹, Jonathan Novoa¹, Thomas J. Nolan², Eduardo Gotuzzo¹, A. Clinton White³¹Instituto de Medicina Tropical 'Alexander von Humboldt' Universidad Peruana Cayetano Heredia, Lima, Peru, ²University of Pennsylvania, Philadelphia, PA, United States, ³Baylor College of Medicine, Houston, TX, United States

The presentation of human strongyloidiasis varies between host populations from a chronic, but limited infection in normal hosts to hyperinfection in patients treated with corticosteroids or with HTLV-1 infection. In murine models, granulocytes (especially eosinophils) and antibody play key roles in controlling infection, but there are few data in human subjects. The objective of this study was to evaluate *Strongyloides* larval antigen specific cytokine responses in strongyloidiasis patients with differing underlying diseases. PBMCs were isolated from 8 newly diagnosed strongyloidiasis patients in Lima, Peru, 3 of them with HTLV-1 co-infection, and 2 uninfected controls. PBMCs were cultured in complete media in the presence or absence of crude *Strongyloides* larval antigen. Supernatants were collected and stored at -80°C until analysis by multiplex bead immunoassay. We observed spontaneous production of the Th1 type cytokines [IFN- γ (414.5 \pm 36 pg/ml) and CXCL-10 (5031 \pm 3295 pg/ml)] and the regulatory cytokine IL-10 (43.5 \pm 29 pg/ml) in unstimulated PBMCs from strongyloidiasis/HTLV-1 co-infected individuals. By contrast, we noted spontaneous production of IL-17 in patients with strongyloidiasis only (27.2 \pm 15.7 pg/ml). With antigen stimulation, patients with

strongyloidiasis-only but not HTLV-1 co-infection produced IL-5 (566.7 \pm 269 pg/ml for those with just strongyloidiasis versus 69.3 \pm 64 pg/ml in those co-infected with HTLV-1) IL-13 antigen-specific response was preserved in both, strongyloidiasis and strongyloidiasis/HTLV-1 co-infected patients (237.6 \pm 98.73 vs 255 \pm 145 pg/ml). In conclusion, we were able to confirm spontaneous Th1 and regulatory cytokine production as reported before in HTLV-1 infected patients. Spontaneous production of IL-17 (involved in neutrophil production and recruitment) and antigen-driven production of IL-5 (involved in eosinophil production) further support the key role of granulocytes in control of strongyloidiasis.

(ACMCI Abstract)

1108

MYELOPEROXIDASE IS REQUIRED FOR PROTECTIVE ADAPTIVE IMMUNITY TO STRONGYLOIDES STERCORALIS IN MICE

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Neutrophil recruitment is required for protective immunity against *Strongyloides stercoralis* infective stage larvae (L3) during both the innate and adaptive immune responses. Additionally, neutrophils are capable of killing L3 *in vivo*, also during both the innate and adaptive immune responses. To determine what neutrophil products are capable of killing L3, the neutrophil granule component myeloperoxidase (MPO) was tested as a representative oxygen-dependent antimicrobial enzyme while neutrophil elastase (NE) was tested as an oxygen-independent antimicrobial enzyme. MPO and NE knock-out (KO) mice were infected with *S. stercoralis* L3, and the ability of the mice to mount effective immune responses was monitored. NE KO and wild-type C57BL/6 mice had similar ability to kill L3 during both the innate and adaptive immune responses. MPO KO mice also exhibited no difference in larval killing versus wild type mice during the innate immune response. However, immunized MPO KO mice had a statistically significant reduction in the number of L3 killed versus wild type controls, despite normal recruitment of cells. These data suggest that neutrophil elastase is not required for the innate or adaptive immune response to *S. stercoralis*, while MPO is required for the killing of L3 by neutrophils in the adaptive response, but not during innate immunity.

(ACMCI Abstract)

1109

POOR SANITATION AND HELMINTH INFECTION PROTECT AGAINST SKIN SENSITIZATION IN VIETNAMESE CHILDREN: A CROSS-SECTIONAL STUDYCarsten Flohr¹, Luc Nguyen Tuyen², Sarah Lewis³, Rupert Quinnell⁴, Truong Tan Minh⁵, Ho Thanh Liem⁶, Jim Campbell¹, David Pritchard⁷, Tran Tinh Hien⁸, Jeremy Farrar¹, Hywel C. Williams⁷, John Britton⁷¹Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam,²Khanh Hoa Provincial Centre for Malaria and Filariasis Control, NhaTrang, Vietnam, ³University of Nottingham, Nottingham, United Kingdom,⁴University of Leeds, Leeds, United Kingdom, ⁵Khanh Hoa Provincial HealthService, Nha Trang, Vietnam, ⁶Khanh Son District Health Service, KhanhSon, Khanh Hoa Province, Vietnam, ⁷Nottingham University, Nottingham,United Kingdom, ⁸Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam

Allergic disease is uncommon in developing countries, especially in rural areas. A protective effect of geohelminth infection, among other environmental factors, has been implicated as a potential explanation. The objective of this study was to determine whether current helminth infection is associated with a reduced prevalence of allergen skin test sensitization in a South-East Asian population of children with a

high prevalence of hookworm infection. All primary and secondary schoolchildren from four neighbouring communes in a rural district of central Vietnam were invited to take part in a cross-sectional survey. Allergen skin sensitization to housedust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), and American cockroach (*Blattella americana*) were measured and stool samples for qualitative and quantitative geohelminth estimation collected. 1,601 children age 6-18 participated. Sensitization to dustmites was present in 14.4%, and to cockroach in 27.6% of children. In univariate analysis, sensitization to either allergen source was less frequent in children with hookworm or *Ascaris* infection, and increased in those with better sanitation, including flush toilets and piped drinking water. In a mutually adjusted model, the risk of sensitization to dustmites was reduced in those with higher hookworm burden (adjusted OR for 350+ versus no eggs per gram=0.61, 95% CI 0.39-0.96), and with *Ascaris* infection (adj OR=0.28, 0.10-0.78), and increased in those using flush toilets (adj OR for flush toilet versus none/bush/pit=2.51, 1.00-6.28). In contrast, sensitization to cockroach was not independently related to geohelminth infection but was increased in those regularly drinking piped or well water rather than from a stream (adj OR=1.33, 1.02-1.75). In conclusion, geohelminth infection, sanitation and water supply influence the risk of allergic sensitization in Vietnamese children. These findings are consistent with a protective effect against allergy by geohelminth or other gastrointestinal infection.

Author Index

A

- Anuegoonpipat, Atchareeya 474
 Aaberge, Ingeborg S. 700
 Aapore, Thomas 692
 Abath, Frederico G. C. 112
 Abbasi, Ibrahim 627, 736
 Abbassy, Magda M. 91
 Abd Elaziz, Khaled 480
 Abdoulaye, Barry 860
 Abdoulaye, Traore 860
 Abdulkhalilova, Gulnara K. 314
 Abel, Jason A. 680
 Abot, Esteban N. 1050, 204, 903
 Abraham, David 1108
 Abbruchah, Harry H. 309
 Achan, Jane 320
 Acholonu, Alex D. W. 501
 Achur, Rajeshwara N. 511
 Acosta, Luz P. 1070, 22, 23, 24, 28, 417
 Acuna-Soto, Rodolfo 780
 Adama, Gansane 197
 Adamou, Abdoulaye 923, 926
 Adams, David P. 36
 Adams, John H. 569, 646
 Addae, Francis 1074
 Addiss, David 349
 Adedapo, Aduragbemi 173
 Adedapo, Aduragbenro D. A. 168, 177, 186
 Adedeji, Ahmed A. 858, 857
 Adedoyin, Olanrewaju T. 86, 31, 85
 Adegbola, Hannah 423
 Adejinmi, Johnson O. 910
 Ademowo, Olusegun G. 152, 173, 722, 161, 177, 186
 Adeyemo, Funmilola C. 186
 Adiambo, Christine 998
 Adimi, Farida 1079
 Adisa, Adetutu R. 161
 Adjalley, Sophie H. 327
 Adjapong, Gloria 407
 Adjei, Andrew A. 851
 Adjei, Ohene 309
 Adjei, Samuel 309
 Adjuik, Martin 559
 Adoke, Yeka 305
 Adriano, Kim 755
 Adungo, Nicholas 285
 Afolabi, Bangmboye 564
 Afrane, Yaw A. 1057
 Agatsuma, Takeshi 132
 Agbor, Jean-Pierre 589
 Aggarwal, Deepak 605
 Aggarwal, Gautam 296
 Aghighi, Zohreh 837
 Agnamey, Patrice 306
 Agtini, Magdarina D. 443
 Agudelo, Henry 555
 Ague-Bales, Jacquelyn 155, 514
 Aguiar, Joao Carlos 1048, 577
 Aguilar, Laura 55
 Aguinaga, Juan G. 712
 Aguirre, Eglys 103, 107, 108
 Aguirre, Marion 456, 458
 Ahikpah, Nguessan 194
 Ahmad, Jamil O. 842
 Ahmed, Loay 633
 Ahn, Sun-Young 153
 Ahorlu, Collins S. K. 199
 Ahoua, Laurence 425
 Ahuja, Vineet 260
 Ajariyakhajorn, Chuanpis 1010, 466
 Ajayi, Ikeoluwapo O. 429
 Akanbi, Olusegun M. 152
 Akanmori, Bartholomew D. 574
 Akech, Samuel 342
 Akhoundi, B 1032
 Akinyi, Sheila 298, 847, 891, 972
 Akishule, Denise 325
 Ako, Beranger A. 194
 Akogun, Oladele B. 427
 Akpogheneta, Onome J. 988
 Akter, Jasmin 380
 Akujobi, Campbell 272
 Al-Buloushi, Adel 56
 Alvarez, Angel 469
 Alakbarova, Saida 963
 Alarcon, Maritza 139, 140
 Alassane Oumar, Aboubacar 860
 Albanis, Effsevia 735
 Alcantara, Leda 1035
 Alcántara, Virginia E. 748
 Alday, Portia 631
 Aldebert, Delphine 205, 210, 30, 355
 Aldstadt, Jared 245
 Aleman, Mireya 823
 Alessiani, Mario 759
 Alexander, Neal 614, 693
 Alford, Lea M. 1058
 Alfred, Tiono B. 197
 Ali, Ibne K. M. 621
 Alifrangis, Michael 172, 448, 551
 Aliota, Matthew T. 239
 Alisjahbana, Bachti 467
 Alker, Alisa P. 550, 658
 Allan, Brian F. 629
 Allan, James C. 413
 Allan, Sandra 248, 38
 Allweiss, Pamela 774
 Almaráz Barrera, Ma. De Jesús 259
 Almela, M^a Jesús 535, 169
 Alonso, Pedro 13, 14, 324, 35, 1001, 674, 764
 Alonso, Wladimir J. 313, 43
 Alphonse, Ouédraogo 1002, 197
 Alto, Barry W. 236, 459
 Alumasa, John N. 185
 Aluvihare, Channa 695
 Alvarez, Angela 528
 Alvarez, Carlos 694, 735
 Alvarez, Maritza 366
 Alvarez Vera, Mayling M. 102, 100, 103, 106, 108, 369, 469, 99
 Amador, Juan Jos^a 367
 Amadou, Konate T. 197
 Amagana, Dolo 206
 Amakye, Joseph S. 396
 Ameke, Christine 325
 Amenga-Etego, Lucas N. 211, 449
 Amerasinghe, Priyanie H. 551
 Ameye, Caroline 543, 855
 Amidou, Diarra 197
 Amidou, Samie 496
 Amimo, Fred 691, 927
 Amin, Omar M. 72
 Amoah, Kwado 309
 Amós, Sonia 1001, 674
 Ampuero, Julia S. 788
 Anand, Setty B. 1025
 Anantapreecha, Surapee 474
 Ananth, Cande V. 32
 Anders, Gerlind 1031
 Anderson, Curtis 797
 Anderson, Jennifer M 741
 Anderson, John F. 237
 Anderson, Jennifer M. 3
 Anderson, Justin R. 727
 Anderson, Lori 21
 Anderson, M. 599
 Anderson, Michael 605
 Anderson, Marc O. 333
 Anderson, Timothy J. C. 377
 Anderson, Wineston 504
 Andersson, Neil 368
 Andrade, Christy 1042
 Andrade, Gabrielle 107
 Andrade, Lucia 81
 Andrade-Narvaez, Fernando J. 690
 André, Deelder M. 732
 Andrews, Katherine T. 669
 Andrews, Simeon 539, 540
 Anglim, Anne M. 440, 47, 701
 Angov, Evelina 576, 849
 Angulo, Carlos 436
 Angulo-Barturen, Iñigo 1004, 528, 529, 530, 535
 Anh, Dang Duc 43
 Aniya, Charmaine S. 983
 Ansah, Nana A. 890
 Anstead, Gregory M. 786, 787
 Anstey, NM 159, 339, 719, 995
 Anthony, Gabriel A. 75
 Anthony, Richard M. 403, 703
 Antia, Meher 163
 Anto, Francis 449, 559
 Antolin, Michael F. 729
 Antonio, Carlos A. 545
 Antonio-Nkondjio, Christophe 916
 Antonjaya, Ungke 439
 Anyorigiya, Thomas 890
 Apollo, Duncan 770, 773
 Aponte, John 1001, 324, 674
 Appawu, Maxwell 242, 398, 65
 Apperson, Charles 364, 917, 605
 Arana, Byron 347
 Arana, Yanina 388
 Arango, Maria del Carmen 103
 Arathoon, Eduardo 825
 Araujo, Sonia 140
 Araujo, Sergio F. 1061
 Araujo-Castillo, Roger V. 424, 93, 94
 Arauz-Ruiz, Patricia 500
 Araz, Engin 941
 Arboleda-Naranjo, Margarita 452
 Areekul, Chutinun 863
 Arenas-Pinto, Alejandro 436, 438
 Arensburger, Peter 227, 910
 Ari, Mary D. 50
 Arias, J. 599
 Arie, Takayuki 996
 Arie, Frederic 550
 Arima, Akihiro 182
 Armah, Henry 149
 Armah, Henry B. 851, 991
 Armbruster, Peter 923
 Armedy, R 719
 Armien, Armien G. 958
 Armien, Blas 823, 827, 958
 Armstrong Schellenberg, Joanna 13
 Arostegui, Jorge 368
 Arrive, Elise 321
 Arruda, Erico A. Gomes. 826
 Arruda, Mercia 934
 Arteaga, Griselda 823
 Arterburn, Jeffery 966
 Artsob, Harvey 665
 Arulogun, Oyedunni 723
 Arvelo, Wences 312, 42, 437
 Ascolillo, Luke 959
 Asgharpour, Amon 384
 Asher, Ludmila V. S. 284
 Ashford, David 1067
 Ashraf, Mohammad 504
 Asnis, Deborah S. 1065, 441, 744, 745, 781
 Assadou, Mahamadoun H. 1100

A-2

Important Note: The number(s) following author names refers to the abstract number.

- Assmar, Mehdi 837
 Astete, Helvio 694
 Athanazio, Daniel 1087
 Atibu, Joseph 32
 Atkinson, Prescott 300
 Atkinson, Peter W. 227, 695, 910, 587
 Atrubin, David 50
 Atugubah, Frank 449, 890, 559
 Atwill, E. Robert 383
 Augustini, Peter 27, 954
 Augustine, Swinburne A. J.. 330
 Augustinova, Andrea 428
 Auliff, Alyson 191
 Aparakkitanon, Saranya 183
 Avila, Guillermina 55
 Avila, Jeanette 792
 Avila, Mario 958
 AvilÃ©s, William 367
 Awadalla, Philip 299
 Awandare, Gordon A. 207, 208, 344, 352, 491, 513, 997
 Awando, Ken 998
 Awono-Ambene, Parfait 916
 Awusabo-Asare, Kofi 317
 Ayala, Aurimar 468
 Ayi, Irene 950
 Ayi, Kodjo 160, 162, 166, 852, 893, 975
 Ayim, Matilda 309
 Ayisi, John 975
 Ayres, Jennifer 891
- B**
- Baba, Duza J. 384
 Babel, Nina 107
 Babelova, Olga 428
 Baber, Ibrahim 1078
 Babino, Zachary 765, 831
 Babu, Subash 1020
 Baby, Mounirou 1100
 Bacellar, Olivia 1030
 Bacellar, OlÃ­via 451
 Bacellar, Olivia 57
 Bach, Cam V. 1005
 Bacon, David 142
 Bacon, David J. 213, 545, 546
 Badaki, Jacqueline A. 427
 Bagayoko, Mamadou W. 165
 Bagayoko, M W. 866
 Bagheri, farideh 837
 Bahnck, Carolyn M. 728
 Baimai, Visut 908
 Bain, Odile 56
 Baird, Kevin 377
 Baisor, Moses 1034
 Bajsa, Joanna 865
 Bajunirwe, Francis 700
 Baker, Murray 564
 Baker, Virginia S. 564
- Baker, William P. 64
 Bakker, Mirjam I. 1064
 Bakshandeh, Arta 772
 Balakathiresan, Nagaraja Sethuraman 695
 Balamurugan, Ramadass 41
 Balderama, Margarita 1031
 Balish, Amanda 756
 Balk, Deborah 955
 Balkan, Suna 425
 Balmaseda, Angel 1094, 111, 367, 368
 Balolong, Ernesto 631
 Balsitis, Scott J. 1092
 Balu, Bharath 569
 Bambal, Ramesh 864
 Bambury, Mark 553
 Banania, Glenna 204, 518, 577, 903
 Banda, Artemisa Ana 674
 Banda, Cesar 1083
 Banda, Patrick 527
 Bandyopadhyay, Kakali 450
 Bandyopadhyay, Nibedita 180
 Bangs, Michael J. 908
 Banguero, Monica 879
 Bannister, Lawrence H. 848
 Baquet, Sophie 425
 Barale, Jean-Christophe 655
 Barban, Veronique 456, 458
 Barbi, Joseph 687, 839, 971
 Bardaji, Azucena 1001, 324, 674
 Barde, Pradip V. 1089
 Bardhan, Pradip K. 702
 Baresch-Bernal, Andrea 1067
 Barillas-Muray, Carolina 923
 Barillas-Mury, Carolina 596, 666
 Barnard, Donald 248
 Barnwell, John W. 190, 298, 301, 521, 524, 534, 840, 844, 845, 848, 198, 203
 Baroni, Pablo 644
 Barrandeguy, MarÃ­a 1043
 Barrera, M. A. 62
 Barreto, Mauricio L. 1009
 Barrett, Alan D. T.. 983
 Barrett, Leah 136, 266, 496
 Barrett, Michael P. 537
 Barron, Eduardo 714
 Barroso, Luis F. 619
 Barry, Ryan K. 97
 Bartholomay, Lyric C. 1041, 120, 228
 Barvir, Dave 797
 Basagoudanavar, Suresh H 570
 BasaÃ±ez, MarÃ­a-Gloria 481
 Basri, Hasan 191
 Bassole, Ismael H. N. 229
 Bastos, Melissa S. 196
 Baton, Luke A. 1077
 Batra, Vijay 541, 867
 Batwala, Vincent 419, 700
 Bausch, Daniel G. 659, 277
- Bautista, Christian T. 133
 Bayoh, Nabie 604
 Beall, Bernard 699
 Beard, Brad 278
 Beard, Suzanne 312
 Beare, Nicholas 340
 Beatty, Barry 230
 Beatty, Mark 468, 813
 Beatty, P. Robert 1092, 1093
 Beatty, Wendy 332
 Beaty, Barry J. 643, 645, 729, 911, 939, 1011, 806
 Beau de Rochars, Madsen 349
 Beauharnais, Carole Anne 136, 496
 Beavogui, Abdoul H. 379
 Becerra-Artiles, Aniuska 798, 800, 805
 Beck, David L. 740
 Beck, Eric T. 911
 Beckett, Charmagne G. 457, 467
 Beerntsen, Brenda T. 572
 Beeson, James G. 357, 669, 986
 Beier, John C. 613, 615
 Beingolea, Luis 792
 Belco, Poudiougou 860
 BÃ©lisle, Patrick 631
 Bell, Deborah 489
 Bell, David 534
 Bell, David J. 308, 418
 Bell, Jeffrey A. 121
 Bellemare, Marie-Jossee 536
 Bellinger, David C. 1070
 Belmonte, Arnel 518, 577
 Belmonte, Maria 518, 577
 Beltran, Manuela 802
 Benavente, Luis E. 9, 1081
 Benca, Juraj 428
 Benedict, Mark Q. 222, 586, 598
 Benitez, JesÃºs A. 784, 77
 Benjamin, Lynette 825
 Bennett, Corey J. 812, 814
 Bennett, Kent S. 179
 Bennett, Shannon N. 401
 Bennuru, Sasisekhar 709
 Beno, Pavol 428
 Benton, Jason P. 222
 Bentzel, David E. 747
 Berenguera, Ana 1001
 Berenguera, Anna 324
 Berg, Tiina 891
 Berghout, Joanne 162
 Bergman, Lawrence W. 519
 Bergmann-Leitner, Elke S. 849
 Bergval, Indra L. 403
 Berhe, Nega 70
 Berkhout, Ben 1051
 Berman, Jonathan 1031
 Berman, LaShondra S. 756
 Bermúdez, Evelyn 862
 Bern, Caryn 1026, 945
 Bernabe, Antonio 1082
 Bernard, Frederic 182
- Bernard, Kristen A. 812, 814
 Bernardo, Lidice 100, 102, 106, 99
 Bernhardt, Scott A. 729
 Bernier, Ulrich 248
 Berrada, Zenda L. 455
 Berrizbeitia, Mariolga 499
 Berthaud, Vladimir 492
 Berthe, Abdoulaye 753
 Besansky, Nora 913
 Besansky, Nora J. 1014, 1017, 18, 229, 589
 Best, Ivan 1066
 Beugre, Elisabeth G. 194
 Bhattacharjee, Apurba K. 184, 335, 869, 873
 Bhattacharya, Sudha 260
 Bhattacharya, Sujit K. 447
 Bhavanandan, Veer P. 511
 Bhutta, Zulfiqar A. 48
 Bian, Guowu 907
 Bienvenu, Anne-Lise 1003
 Bienvenu, Sirima S. 197
 Bierkens, Mark F. P. 919
 Bikowski, Margaret 1067
 Bimal, Raajeewa 415
 Bimal, Sanjiva 415, 447, 626
 Bimal, Sanjiv B. 494
 Bin hazim, Awadh 492
 Bird, Michele 1086
 Birren, Ewan 234, 931
 Birren, Bruce 1, 326
 Birren, Bruce W. 1016, 227
 Bishoop, Henry 312
 Bishop, Henry 1037, 433
 Bissagnene, E 855
 Bixby, Lisa M. 505
 Bjorkman, Anders 755
 Bjornstad, Ottar N. 661
 Black, William 230
 Black, William C. 1011, 17, 645, 729, 795, 939
 Blackley, Shanley D. 105
 Blackwell, Jenefer M. 1061
 Blain, Craig 657
 Blair, Carol D. 643, 729, 911, 645
 Blair, Patrick J. 1088, 420, 439, 467
 Blaney, Joseph 982
 Blaney, Joseph E. 981
 Blangero, John 1027
 Blanton, Ronald E. 1009
 Blauvelt, Carla P. 1020
 Blazes, David L. 275, 406, 424, 73, 93, 94, 92
 Blitvich, Bradley 645, 1041
 Blitvich, Bradley J. 911
 Block, Karla 104
 Bloland, Peter B. 10, 1075, 670, 671
 Blomgren, Anna 170
 Blomhoff, Rune 70
 Blood, Melinda 323

- Blum, Johannes A. 1029
 Blum, Lauren S. 42
 Blumstein, Norbert 751
 Boakye, Daniel A. 1074, 396, 45, 398, 65, 91
 Boamah, Daniel -. -. 950
 Bob, Ndeye 977
 Bockarie, Moses J. 1034, 252, 33, 346, 377
 Bodhidatta, L 286
 Boehmer, Gabriel 1101
 Boele van Hensbroek, Michael 88
 Bohle, Scott D. 536
 Boisvert, M. 599
 Boivin, Michael J. 520
 Bojang, Kalifa A. 988
 Bolaños, Claudia 879
 Bolarte, Jose 792
 Bomberger Brown, Mary 280, 282
 Böni, Jürg 319
 Bonnemay, Radana 436, 438
 Bonnet, Maryline 425
 Boonnak, Kobporn 1091
 Boonpradit, Pornsiri 474
 Boonti, Thum 247
 Booth, Mark 553
 Boppana, Venkata D. 39
 Borges, Rafael 139
 Borja-Cabrera, Gulnara 507
 Borrmann, Steffen 755
 Borrow, Ray 700
 Bosch, Irene 798, 799, 800
 Bosch, Norma 798, 800
 Bosio, Christopher F. 939, 960
 Bosio, Chris F. 965
 Bosompem, Kwabena M. 950
 Bossin, Hervé C. 222
 Bottazzi, Maria E. 823
 Boubacar, Togo 860
 Boudko, Dmitri Y. 15, 585
 Bourque, Daniel 746
 Bowen, Anna 312, 437
 Bowen, Richard A. 1042, 815
 Bowman, Natalie M. 1026
 Boye, Alex 1074
 Boyle, Michelle 669
 Boyom, Fabrice F. 181
 Brackney, Doug E. 593, 918
 Braden, Zachery 278
 Bradley, David J. 195
 Bradley, Mark A. 480
 Braga, Érika M. 196
 Branch, OraLee H. 886, 897, 900
 Branco, Luis 277
 Brandt, Mary 825
 Brandt, Stephanie 596
 Bras, Jacques L. 550
 Bresseur, Philippe 306, 307
 Brault, Aaron C. 1042
 Brawn, Jeffrey D. 932, 938
 Brechet, Damien 807
 Breiman, Rob 633
 Bretzel, Gisela 44
 Brewer, Christina M. 121
 Brian, Ward 824
 Bridges, Michael 292
 Brindley, Paul J. 953
 Britch, Seth C. 254
 Brito, Carlos A. A. 796
 Brittain, Nathaniel J. 996
 Brittenham, Gary M. 992
 Britton, John 1109
 Bronner, Yvonne 1105
 Bronzan, Rachel 557
 Bronzan, Rachel N. 340
 Brooke, Basil D. 584
 Brooker, Simon 1068, 1103, 422
 Brousse, Valentine 516
 Brown, Andrew 209
 Brown, Ashley N. 814
 Brown, Charles 242
 Brown, Charles R. 280, 281, 282
 Brown, Evan 850
 Brown, Graham 357
 Brown, Heidi E. 937
 Brown, Jessica 20
 Brown, Joe 318
 Brown, Mary Ann 489
 Brown, Mark R. 922
 Brown, Nick 434
 Brown, Truman R. 992
 Brown, Vincent 425
 Brown, William M. 938, 932
 Brown Kunin, Sharon 769
 Bruder, Joseph T. 1050, 577, 899
 Bruggner, Robert V. 234, 931
 Brunetti, Enrico 1036, 431, 759, 760
 Bruni, Laia 674
 Brutus, Laurent 488
 Bryanseva, Eylena 961, 963
 Bryce, Gary 890
 Buathong, Nillawan 421, 87
 Bubb, Martin 534
 Bubis, Jose 499
 Buezo, Silvia R. 413
 Buffet, Pierre 516
 Bugbee, L. 599
 Bujard, Hermann 203
 Bukirwa, Hasifa 305, 721
 Bunga, Wence 123
 Bungiro, Richard D. 968
 Bunrasri, Chalerm 87
 Burdick, John 64
 Burgess, Steven 539, 540
 Burgess, Timothy H. 804, 979
 Burke, Barbara 1033
 Burke, Donald S. 659
 Burns, James M. 519, 565, 901
 Burri, Christian 1029
 Bustamante, Juan M. 505
 Bustamante, Patricia 555
 Bustos, Javier A. 389, 391, 393, 412, 712
 Buszin, Justin 317
 Butler, William 878
 Butman, Bryan T. 1050
 Buttaro, Caitlin J. 483
 Buzetti, Wilma A. S. 498
 Buzzar, Marcia 1087
 Byarugaba, Justus 341
 Bygbjerg, Ib C. 172, 448, 551
 Byrd, Brian D. 476, 602
 Byrne, Timothy 669
- C**
- C., Anumudu 561
 Cabello de Quintana, Maritza 366
 Cabezas, Cesar 109, 116, 545, 694
 Cabezas, Miriam 335
 Cable, Ritchard G. 264
 Cabrera, Lilia 1026, 1082, 381, 624, 752, 945, 948
 Cabrera, Rufino 792
 Cabrera-Pedroza, Alfredo U. 113
 Cabrera-Mora, Monica 1049, 842, 847, 891, 902, 972
 Caccione, Adalgisa 607, 916
 Caceda, Edna R. 635
 Caceda, Roxana 767
 Caceres, Carlos F. 138, 46
 Cáceres, Omar 109
 Cahill, John D. 63
 Cai, Cindy 97
 Calderon, Carmen 714
 Calderón, Maritza 568, 914
 Calderon-Arguedas, Olger 613
 Calderwood, Stephen B. 1067, 290, 291
 Callahan, Micheal V. 31
 Callahan, Johnny D. 457
 Callahan, Michael 423, 86
 Callahan, M V. 85
 Calvo-Calle, Mauricio 1101
 Calzada, Naifi 101
 Calzetta, Maria 607
 Cama, Vitaliano A. 381, 624, 948, 945
 Camacho, Daria E. 366
 Camacho, Minerva 114
 Camacho-Nuez, Minerva 1011
 Camara, Guimba 502
 Campbell, Corey L. 224
 Campbell, Gerald A. 637, 681
 Campbell, Jim 1109, 287
 Campbell, James D. 1084, 1085, 283, 753, 757
 Campbell, Kathryn S. 234, 931
 Campo, Joseph J. 1050, 899
 Canal, Enrique 791
 Canales, Marco 735
 Canh, Do Gia 43
 Canilena, Louis 338
 Cao, Long 1024
 Cao, Zhifang 1096, 1097
 Cao-Lormeau, Van-Mai 460
 Cappello, Michael 489, 968
 Caputo, Beniamino 231
 Carabin, Hélène 631
 Carcamo, Alvaro 368
 Cardenas, Gabriel 615
 Cardona-Castro, Nora M. 452
 Cardoso, Marly A. 196
 Caridha, Diana 335, 873
 Carlson, Elaine J. 673
 Carlson, Tracy 839
 Carnevale, Eric P. 881
 Carollo, Maria C. C. 450
 Carr, Jean K. 133
 Carrara, Giancarlo 607
 Carrillo-Farga, Joaquin 53
 Carrion, Gladys 135
 Carroll, Darin S. 680, 636
 Carroll, Ryan 515
 Carthew, Tracy 170
 Carvalho, Eunice B. 399
 Carvalho, Edgar M. 1030, 1035, 451, 57, 788
 Carvalho, Luzia H. 196
 Carvalho, Lucas P. 451
 Carvalho, Maria da Gloria 699
 Casapia, Martin 1104
 Cash-Goldwasser, Shama 232
 Cashman, Kathleen 277
 Caskey, Marina F. 683
 Caskey, Susan 937
 Casseb, Samir M. 962
 Cassone, Bryan J. 1014, 18
 Castellanos, Alejandro 263
 Castellanos, Yinet 101
 Castillo, Juan A. 827
 Castillo, Rosa 406
 Castillo, Roger 462, 767
 Castro, Bronislawa 1087
 Castro, Elena 113
 Castro, Erika 828
 Castro, Jorge 106
 Castro, Juan C. 914
 Castro, Jesuina M. 1009
 Catteruccia, Flaminia 222
 Catú, Eduard 347
 Caugant, Dominique A. 700
 Causer, Louise M. 670, 671
 Cavasini, Carlos E. 196
 Cavero, Carlos A. 752
 Caviedes, Luz 1082
 Ceballos-Olvera, Ivonne 1090
 Cecere, María C. 739
 Celermajer, D 339, 995
 Celum, Connie L. 133
 Cerávoló, Isabela P. 196
 Cerna, Luis 929
 Certad, Gabriela 436, 438
 Certain, Laura K. 193

A-4

Important Note: The number(s) following author names refers to the abstract number.

- Cha, Je-Eun 153
 Chabalgoity, Alejandro 714
 Chacaltana, Jesus 406
 Chadee, David 251, 246
 Chagomerana, Maganizo B. 884, 340
 Chai, Jong-Yil 1080
 Chai, Wengang 669
 Chaisawang, Chaisombat 87
 Chakravarty, Sumana 1053, 353
 Chalwe, Victor 432
 Champagne, Donald 2
 Chan, Teik-Chye 365
 Chanama, Sumalee 474
 Chancafe, Jorge A. 791
 Chand, Krisin 191
 Chanda, Pascalina 525, 527, 675
 Chang, Chun C. 129
 Chang, David 980
 Chang, Wonsuk 526
 Chao, Chien-Chung 365
 Chapilliquen, Fernando 929
 Chapman, Lauren J. 629
 Chapola, Erica 1087
 Chaponda, Marjorie 414
 Chapoomran, Soebsakul 183
 Charlab, Rosane 891
 Charlebois, Edwin D. 320
 Charles, Bruce 170, 171
 Charles E, Taylor 606
 Charoenkorn, Malee 863
 Charoenviriyaphap, Theeraphap 247
 Charoenvit, Yupin 204
 Chatsuwana, Tanittha 124
 Chattopadhyay, Suchismita 365
 Chau, Nguyen Van Vinh 1008
 Chau, Tran Nguyen Bich 1008
 Chaudhri, Farida 744
 Chaudhuri, Asok 522
 Chavasse, Des 434
 Chavez-Salinas, Salvador 1090
 Checchi, Francesco 425
 Checkley, Lisa 657
 Cheikh, Dah O. 663
 Chelbi, Ifhem 6
 Chen, A. 292
 Chen, C C. 228
 Chen, Cheng C. 239
 Chen, Huiyuan 1095
 Chen, JunHu 632
 Chen, Lan 104
 Chen, Lin H. 79
 Chen, Peixin 217
 Chen, Shirley C. 361
 Chen, Tom 1096, 217
 Chen, Wei-June 117
 Chen, Xiaoguang 225
 Chen, Zhihui 1097
 Chen, ZhongQiang 647
 Chenault, V. Michelle 395
 Chenet, Stella M. 213
 Cheng, Qin 358
 Chenine, Agnes-Laurence 27, 954
 Chero, Juan C. 392, 393
 Chesson, Joanne 357, 669
 Chiang, Jannifer O. 196
 Chiarello, Anna 366, 862
 Chilengi, Roma 432
 Ching, Wei-Mei 365
 Chinnawirotpisan, Piyawan 371
 Chippaux, Jean-Philippe 488
 Chipungu, Geoffrey 88
 Chirachariyavej, Thamrong 183
 Chirgwin, Sharon R. 818
 Chiroque, Juana 791, 929
 Chisenhall, Daniel 810
 Chitah, Bona 527
 Cho, Pyo Yun 270
 Cho, Shin Hyeong 258
 Cho, Seung-Yull 790
 Choi, Min-Ho 790
 Cholera, Rushina 996
 Choong, Ee Ken 669
 Chotivanich, Kesinee 151
 Chretien, Jean-Paul 878, 998
 Chrigwin, Sharon 710
 Christensen, Bruce M. 120, 228, 239, 348
 Chu, Yong-Kyu 964, 966
 Chuang, Ching-Kai 117
 Chukwuocha, Uchechukwu M. C. 127
 Chung, Dong Hoon 966
 Chung, Su-Fang 808
 Chuquiyauri, Raul 746
 Churcher, Thomas S. 481
 Chusak, P 370
 Cirimotich, Chris M. 249
 Cisse, Moustafa 306
 Cissé, M 331
 Cisse, Moumine 502
 Cissé, M 866
 Cissé, Ousmane H. 304
 Cissé, O H. 866
 Clain, Jérôme 297
 Clark, Anne B. 940
 Clark, Gary 248
 Clark, John M. 726
 Clark, Kyra P. 405
 Clark, Robert L. 182
 Clark, Thomas A. 50
 Clark, Tamara D. 343, 552, 717
 Clarke, Sian E. 422
 Clavel, I 823
 Clayton, April 620
 Clemens, J. D. 443
 Clements, David E. 983, 1045, 980
 Clendenes, Martín 116
 Clennon, Julie A. 737
 Coates, Thomas J. 138, 46
 Cobblah, Millicent 242
 Cockburn, Ian A. 1053, 353
 Cockrill, Jennifer A. 1052
 Coetzee, Maureen 1060, 584
 Cohen, Justin M. 34
 Cohen, Valery 436
 Cok, Jaime 391
 Colborn, James M. 165, 304
 Coldren, Rodney 87
 Coldren, Rodney L. 285
 Cole-Tobian, Jennifer 509, 668
 Coleman, Russell E. 7
 Coleman, Sharon U. 710, 818
 Colina, Olga 142, 545
 Collaborative HIV/STD Prevention Trial Group, NIMH 138, 46
 Coller, Beth-Ann 1045, 980, 983
 Colley, Daniel 969
 Collier, Travis C. 1059
 Collins, Frank H. 1, 1016, 1017, 931, 232, 234
 Collins, Francis H. 227
 Collins, Matthew H. 684
 Coloma, Josefina 368
 Colombani, Sophie 458
 Colombia, Ravreda 879
 Coluzzi, Mario 231
 Comach, Guillermo A. 366
 Conetta, Rick 744, 745
 Conlan, Andrew C. K. 661
 Conn, Jan E. 238, 915
 Connolly, Joseph D. 181
 Connor, Bradley A. 276
 Conover, Craig 278
 Conrad, Patricia A. 383
 Conteh, Solomon 204
 Conway, David J. 219, 231, 988
 Cooke, Brian 854
 Cooney, Christine 743
 Cooper, Roland A. 189, 548, 652, 653, 657
 Copeland, Curtis C. 789
 Coppel, Ross 854
 Coraspe, Virginia 442, 444, 68, 74
 Corbett, Kevin C. 333
 Cordeiro, Marli T. 796
 Córdoba, Freddy 555
 Cordova, Eleazar 1026
 Córdoba, Juliana 914
 Corena, Maria del Pilar 16
 Cornejo del Carpio, Juan 1026
 Cornel, Anthony J. 594
 Corpuz, Gloria 1045
 Correa-Oliveira, Rodrigo 1027
 Corredor, Vladimir 298, 302
 Cortes, Alfred 357
 Cortes, Edison 873
 Cosio, Gabriela 852
 Costa, Emiliana P. A. 463
 Costantini, Carlo 229, 589
 Costantini, Daniel 978
 Coulibaly, A 866
 Coulibaly, cheick Amadou 741
 Coulibaly, Drissa 200
 Coulibaly, Karim 502
 Coulibaly, Mamadou 1017
 Coulibaly, Siaka 125
 Coulibaly, Yaya I. 125, 351, 484
 Coulson, Patricia 734
 Cousin, Marc 755
 Coutinho, Bruna 949
 Coutinho, Bruna P. 944
 Coutinho, Hannah 28, 417
 Coutinho, Hannah M. 1070, 23, 24
 Cowman, Alan F. 986
 Cox, Jonathan 237
 Cox, Josephine H. 146
 Craft, J C. 541, 867
 Craig, Philip S. 711
 Craig, P. S. 715
 Crasta, Oswald 384
 Crawford, Jacob 923
 Creek, Tracy 312, 437
 Crevat, Denis 807
 Crisanti, Andrea 222
 Crockett, Maryanne 160, 166, 567
 Croft, Donita 278
 Crosby, Seth D. 705, 706
 Crowley, Michael R. 897, 900
 Crudder, Chris 422
 Cruz, Christopher 767
 Cruz, Guillermo 469
 Cruz-Ortiz, Nancy 347
 Cubero, Javier 735
 Cuffee, P. 599
 Cui, Liwang 571, 898, 904
 Cui, Long 571
 Cummings, Derek A. T. 470
 Cupp, Eddie W. 611
 Curns, Aaron 278

D

- D'alessandro, Umberto 307, 875
 D'Arcadia, Rosane R. 196
 D'Souza, Martin J. 749
 da Silva, Alexandre J. 1037, 141, 385, 433, 450, 453, 758, 312
 da Silva, Andréa Nazaré M. R. 112
 da Silva-Nunes, Mônica 196
 Dabire, Roch 724
 Dabis, Francois 321
 Dabritz, Haydee A. 383
 Dac Cam, Phung 1086
 Dada-Adegbola, Hanah O. 86, 85
 Dagoro, Henry 1034
 Dail, Kathy 364
 Daily, Johanna P. 303, 326
 Daly, Thomas M. 519
 Dama, Souleymane 379
 Damon, Inger K. 680, 278, 636
 Dang, Hoang M. 1005

- Daniel-Ribeiro, Claudio T. 198
 Daniels, Simone 50
 Danson, F. M. 715
 Dantoine, François 425
 Dao, Adama 591, 923, 926
 Daou, Modibo 892
 Daouda, Diallo 192
 Dapkiewicz, Rebecca 807
 Dara, Antoine 379
 Dara, Niawalou 856
 DaRe, Jeana T. 378, 881
 Darling, Samuel T. 608
 Darwish, Magdi 633
 Das, Pradeep D. 446, 415, 447, 626
 Das, Vidya N. Ravi. 447
 Dasch, Gregory A. 1039, 1065
 Dass, Krisha 416
 Daszak, Peter 477, 955
 Davenport, Gregory C. 207, 344, 352, 491, 513
 David, Catarina 674
 David, Mak 155
 David, Peter 516
 Davidson, Whitney 278
 Davis, Charles B. 864, 530
 Davis, Jeffrey P. 278
 Davis, Margaret 312, 437
 Davis, Richard 701
 Davis, Stephanie E. 851
 Davis, Timothy 993
 Davis-Rivers, Andrea N. 943, 944
 Dawson, Jennifer 940
 Day, Nicholas P. J. 151
 Day, Richard D. 156
 de Almeida, Marcos E. 141
 de Bosch, Norma 805
 De Brito, Thales 81
 De Donato, Marcos 828, 885
 de Gruijl, Tanja 1051
 de Jesus, Amélia R. 451
 de Jonge, H.C.C. 1062
 de Souza, Dziejdom K. 1074, 396, 45, 398
 de Vries, Henry J. 497
 de Vries, Peter J. 497
 De-Simone, Salvatore G. 198
 Debebe, Zufan 853
 deBruyn, Becky 612
 DeCaprio, David 326
 DeGaetano, Art 243
 Deitsch, Kirk 978
 DeJong, Randy 923
 del Angel, Rosa M. 1090, 794
 del Reino, Paloma 169, 535
 DeLaPaz, Robert L. 992
 Delgado, Bonnibel 269
 Delgado, Olinda 442, 444, 68, 74
 Delgado, Walter 488
 della Torre, Alessandra 1014
 della Torre, Alessandra 229, 231, 256, 589, 607
 Dembelé, Benoit 125, 351, 484
 Dembélé, Massitan 484
 Deng, Bingbing 657
 Dengue Group, CIETNicaragua 368
 Deniz, Ahmet 957
 Dennull, Richard A. 871, 873
 Dent, Arlene E. 882
 deOliveira, Ana 293
 DeRisi, Joseph L. 333, 999
 Derr, Alan 326
 Desai, Manish A. 905
 deSavigny, Don 434
 Devine, Gregor J. 608, 694
 Dewar, Vincent 274
 Dewasthaly, Shailesh 984
 Dhondt, Andre 477
 Di John, David 441, 781
 Dia, Ibrahima 231, 607
 Diabate, Abdoulaye 724, 923
 Diallo, Abdoumbaki 856
 Diallo, Abdallah A. 351, 484, 125
 Diallo, Boubacar 663
 Diallo, Dapa A. 1100, 200, 351
 Diallo, Mouhamadou 355
 Diallo, Mawlouth 663
 Diallo, Mamadou A. 205
 Diallo, Souleymane 1084, 1085, 698, 757
 Dialynas, Emmanuel 234, 931
 Diarra, Issa 892
 Diarra, R. A. 866
 Dias, Gutemberg H. 1061
 Dias, Juarez P. 1009
 Dias, Marcia C. F. S. 1061
 Diassiti, Angelina 567
 Diawara, Sory I. 1100
 Diaz, Victor 488
 Diaz-Badillo, Alvaro 1011, 230
 Dickerson, Tobin J. 122, 819
 Dickherber, Megan L. 842, 902, 891
 Dicko, Alassane 1100, 200, 856
 Dicko, Yahia T. 856
 Die-Kakou, H 855
 Diène Sarr, Fatoumata 30
 Dietrich, Gabrielle 2
 DiFedele, Lisa M. 489
 Dillingham, Rebecca 136, 496
 Dillon, Michael 680
 Dimopoulos, George 1013, 1077, 907
 Ding, Jinhui 548
 Dinglasan, Rhoel R. 667
 Dinsmore, Jonathan H. 799
 Diop, Bernard 977
 Diop, Djibril 663
 Diop, Ousmane M. 663
 Diouf, Ababacar 1099
 Direny, Abdel 349
 Diuk-Wasser, Maria A. 937
 Djibo, Ali 661
 Djimde, Abdoulaye 200
 Djimde, Abdoulaye A. 379
 Dluzewski, Anton R. 848
 Do, Ha Q. 465
 Dobardzic, Rechad 822, 822, 490
 Dobler, Gerhard 44
 Dobson, Andrew P. 477
 Dodean, Rosie 336, 337, 870, 872
 Doodoo, Daniel 574, 890
 Dokomajilar, Christian 373, 375, 673
 Dolan, Marc 2
 Dolecek, Christiane 1008, 1086, 287
 Doll, Michelle 295
 Dolo, Amagana 1100
 Dolo, Guimogo 591, 1078
 Dominguez-Alpizar, Jose Luis 54
 Don, Mark 637
 Dondji, Blaise 968
 Donelson, John E. 649, 685, 838
 Dong, Tao 1006
 Dong, Yuemei 1013, 1077
 Donis, Ruben 1088
 Donkor, Delali 242
 Donnelly, Martin J. 607, 697, 696
 Doolan, Denise L. 1050, 221, 577, 899, 204, 903
 Dore, Roberto 760
 Dorkenoo, Monique 482
 Dorn, Patricia 1028
 Dorregaray, Ruben 412
 Dorsey, Grant 1000, 305, 307, 343, 375, 377, 552, 672, 673, 717, 320
 Dotson, Ellen M. 738
 Doumbia, Mama N. 1084, 1085, 698, 757
 Doumbia, Seydou 1078, 484, 502, 594, 741
 Doumbo, Ogobara K. 1100, 200, 351, 379, 856, 892
 Doung, Socheat 550
 Dow, Geoffrey S. 335
 Dowell, Scott F. 49
 Dowell, Scott L. 1039
 Dozie, Ikechukwu N. S. 126
 Drazba, Judith A. 648
 Drosten, Christian 664
 Drysdale, Melissa 1067
 Duan, Junhui 299
 Dubray, Christine 661
 Duffull, S 339, 995
 Duffy, Michael F. 669
 Duffy, Patrick E. 356, 850
 Duggal, Priya 380
 Duggan, Peter 578
 Duke, Stephen O. 865
 Dumonteil, Eric 397, 507, 508, 836
 Dunbar, Abram 501
 Duncan, Elizabeth H. 849
 Duncan, Mark 832
 Duncan, Robert 615
 Dung, Nguyen Thi Phuong 1008
 Dunk, David 292
 Dunn, John 936
 Dunne, David W. 26
 Dunyo, Sam K. 754
 Duparc, Stephan 180
 Dupnik, Kathryn 136, 1061
 Dupuis, Alan 1040
 Dupuis II, Alan P. 812
 Duran, Salomon 116
 Durand, Solomon 545
 Durant, Lisa 766, 830
 Durbin, Anna P. 981, 1094
 Durbin, Joan 687
 Duriseti, Sai 157
 Duriyaphan, Pochaman 445
 Dusfour, Isabelle 590, 908
 Dutra, Walderez O. 1030, 451
 Dvorak, James A. 996
 Dyer, Jessie 810
 Dzikowski, Ron 978
 Dziuba, Natallia 681
-
- E**
- Eampokalap, Boonchuay 49
 Earhart, Ken 633
 Earl, Long 813
 Easterbrook, Judith D. 677, 678, 80
 Ebel, Gregory D. 1040
 Eberhard, Mark 433, 56
 Ebihara, Hideki 660, 665
 Ebsworth, EP 719
 Echenagucia, Marion 805
 Edelman, Robert 1101
 Edillo, F. E. 599
 Edman, John D. 726
 Edmund, Seto 1073
 Edstein, Michael 170, 171
 Edwards, Eric A. 280, 281, 282
 Edwards, Michael 832
 Edwards, Rosemary 134, 69
 Edwards, Tansy 754
 Egah, Daniel 564
 Egas, Josefina 771
 Egyir, Beverly 242
 Ehrenkauffer, Gretchen M. 621, 384
 Eigege, Abel 1069
 Eisen, Damon 358
 Eisenberg, Joseph N. S. 316, 400, 905
 Ekala, Marie Thèsrère 977
 Ekanayake, Sajeewane M. 457
 Eke-Njoku, John 67
 Eko, Francis O. 149
 Ekoue-Kovi, Kekeli A. 185
 El Meneza, Safaa A. N. 408
 El Ridi, Rashika A. F. 731, 66
 El Setouhy, Maged 480
 El-Sayed, Nasr 60

A-6

Important Note: The number(s) following author names refers to the abstract number.

Eldin De Pecoulas, Philippe 306
 Elish, Diana 744
 Elliott, Alison M. 26, 325, 553
 Ellis, Brett R. 924
 Ellis, Michael W. 405
 Ellis, Ruth D. 1100, 421
 Elyan, Diaa 633
 Elyazar, Iqbal 191
 Emerson, Ginny 636
 Emukah, Emmanuel C. 78
 Endo, Yaeta 1047, 1048
 Endy, T P. 370
 Enevold, Anders 172
 Engber, Barry 364
 Engel, Jürgen 1031
 Engel, Jeffrey 364
 Engering, Anneke J. 218, 566
 English, Mike 720
 Ennis, Francis A. 1044
 Ennis, F A. 370
 Enria, Delia A. 1043, 644
 Enyong, Peter 122
 Erdman, Laura 852
 Eremeeva, Marina E. 1065
 Ergonul, Onder 957
 Erhart, Annette 875
 Erickson, John 1102
 Erickson, Sara M. 348
 Ernest, Samuel K. 86, 423
 Ernst, Kacey C. 34, 883, 888
 Erttmann, Klaus D. 817
 Escalante, Ananias A. 188, 190
 Escobar, Francys 862
 Escobar, Humberto 555
 Escobedo, Karin 694
 Escudero, Juan 792
 Espino, Ana M. 267, 268, 269
 Espino, Fe Esperanza 933
 Espinosa, Benjamin J. 406
 Espinoza, Angélica 767
 Espinoza, Jose R. 735
 Espinoza- Gómez, Francisco 113, 144
 Espitia, Claudia 650
 Esposito, Marco 618
 Esquinca-Avilés, Héctor A. 1011
 Essbauer, Sandra 44
 Estambale, Benson 422
 Estes, Mark D. 681
 Estrella, Bertha 771
 Ettinger, Nicholas A. 990
 Evans, Amy M. 806
 Evans, Clive 384
 Evans, Carlton A. 1082, 1083
 Evans, James 798
 Evans, Sandra R. 97
 Eversole, Rob 123
 Evidente, Antonio 865
 Ewetola, Raimi 36
 Ewing, Daniel 104, 804
 Eyma, Etna 496

F

Fabbri, Cintia M. 644, 1043
 Faber, William R. 497
 Fabiosa, Flor G. 1041
 Facchinelli, Luca 256
 Fadare, Joseph O. 173
 Fadeel, Moustafa A. 60
 Fagonde, Everton 81
 Fair, Joseph N. 277
 Fairhurst, Rick M. 996
 Fakhoury-Makki, Rajaa 150
 Falade, Adegoke 31
 Falade, Catherine O. 31, 429, 722, 723, 85, 86, 186, 173, 177, 423
 Fallon, John M. 798
 Falzarano, Darryl 660
 Fan, Chia K. 129
 Fandeur, Thierry 550
 Farah, Idle 734
 Farfan, Marilu 388
 Farfan-Ale, Jose A. 729
 Farias, Dani R. 1030
 Farias, Kleber J. S. 115
 Farid, Hoda A. 480
 Farnon, Eileen 198
 Farrar, Jeremy 1005, 1006, 1007, 1008, 1109
 Faruque, Abu S. G. 702
 Fasabi Espinar, Manuel 746
 Fasendini, Bernardo 644
 Fateye, B A. 858, 857
 Faucher, Jean-François 306
 Fay, Michael 351
 Faye, Ousmane 502, 663
 Febles, Taynet 441, 781
 Fedanov, Andrew 521
 Fegan, Greg 342
 Fei, Zhangjun 384
 Feldmann, Friederike 660
 Feldmann, Heinz 660, 665
 Feleke, Getachew 131, 742
 Felices, Vidal 634
 Feng, Zheng 790
 Fenwick, Alan 1068, 952
 Ferdig, Michael T. 653, 657
 Ferguson, Heather 1054, 1055
 Fermon, Florence 661
 Fernandez, Fernando 1043
 Fernandez, Facundo 311
 Fernandez-Pol, Sebastian 515
 Fernandez-Salas, Ildefonso 729
 Fernando, Lisa 660
 Ferrandiz, Josette 1003
 Ferrara, Giuseppe 436, 438
 Ferrari, Matthew J. 661
 Ferreira, Marcelo U. 196, 328
 Ferrell, Robert R. 207, 352
 Ferrer, Maria V. 62
 Ferrer, Santiago 1004, 530, 864, 864
 Ferrer-Rodriguez, Ivan 880

Ferro, Philip 277
 Fidock, David A. 327, 647
 Field, Ann E. 989
 Fiestas, Victor 116
 Fievet-Groyne, Françoise 807
 Fikrig, Erol 237
 Filice, Carlo 1036, 431, 759, 760
 Fincham, John E. 1106
 Finkbeiner, Thomas 312, 437
 Firbas, Christa 985
 Fischer, Julie E. 49
 Fischer, Kerstin 128, 348
 Fischer, Peter 128, 348, 704
 Fischer, Philip R. 95
 Fish, Durland 937
 Fisher, Julia K. 563
 Fisk, Tamara L. 1039
 Fitzgerald, Daniel W. 136, 496
 Fitzpatrick, Meagan 1017
 Flanagan, Brigitte A. 64
 Flanagan, Joseph 449
 Flannery, Luciana 902
 Fleckenstein, Lawrence 761
 Flegler, Carol 292
 Flisser, Ana 387, 53, 54, 55
 Flohr, Carsten 1109
 Flores, M 929
 Flores-Mendoza, Carmen 935
 Florey, Lia S. 1072
 Flyer, Johanna G. 361
 Fogg, Carole 419, 700
 Folarin, Onikepe A. 187
 Foley, Janet E. 96, 98
 Foley, Patrick 96, 98
 Fonseca, Benedito A. L. 115, 463, 471
 Fonseca, Dina M. 728, 253
 Fontenille, Didier 229, 589
 Foppa, Ivo M. 610, 599, 928
 Ford, Karen 564
 Ford, Robert 564
 Fortes, Filomeno 607
 Foster, Jerome 281, 282
 Fotouhi, A. 1032
 Fox, LeAnne M. 145
 Fox, Matthew P. 145
 Foy, Brian D. 224, 593, 918, 925
 Frace, Mike 636
 Frame, Laura 922
 Frances, Stephen P. 233
 Franco, Pia 406
 Franco-Paredes, Carlos 143, 442, 444, 62, 68, 74, 784
 Frank, Matthias 978
 Franz, Alexander W. E.. 725, 597
 Fraser, Malcolm J. 1089
 Frazar, Christian 453
 Frederick, Lafayette 1105
 Frempong, Kwadwo K. 242
 Fridkin, Scott 825
 Fried, Michal 356, 850
 Friedland, Jon S. 1082, 1083

Friedman, Jennifer F. 1070, 1070, 24, 417, 22, 23, 28
 Friedman, Scott L. 735
 Frøholm, Oddvar 700
 Frolov, Ilya 637, 638, 640, 681
 Fryauff, David F. 65
 Fryauff, David J. 377, 449, 559
 Fryauff, Michael J. 361
 Fuchs, Jeremy F. 120, 228, 239, 348
 Fuenmayor, César 776, 778, 779
 Fuentes, Luis 792
 Fujioka, Hisashi 648
 Fukuda, Mark M. 218, 416, 421, 566, 374, 87
 Fuller, Douglas O. 613
 Fuller, Matt S. 476
 Fumadó, Victoria 426
 Fumoux, Francis 329
 Fursa, Isaac B. 1033
 Furtado, Patricia B. 112
 Furuya, Tetsuya 548, 571

G

Gabitzsch, Elizabeth 2
 Gaestel, Matthias 512
 Gaetant, Engleed 134
 Gaines, D. 599
 Gaines, David 928
 Gajurel, Kiran 131
 Galagan, James E. 326
 Galappaththy, Gawrie N. L. 551
 Galardo, Allan K. 934
 Galinski, Mary R. 1049, 198, 298, 301, 302, 521, 524, 840, 842, 844, 845, 847, 848, 891, 972
 Galloway, Renee 50
 Galvan, Rosa 133
 Gamain, Benoit 510
 Gamboa, Dionicia 886
 Gamboa-Leon, Rubi 507
 Gamston, Courtney 1012, 580
 Gan, Weiniu 891
 Ganeshan, Harini 1050, 204, 903
 Gangemi, David 797
 Ganley-Leal, Lisa M. 969
 Ganoza, Christian 568
 Gansane, Adama 877
 Gaona, Heather W. 869
 Gaona, Jenny 298
 García, Adolfo 530
 García, Anapatricia 847
 Garcia, Gissel 103, 107, 108
 Garcia, Hugo 410
 Garcia, Hector H. 388, 389, 390, 391, 392, 393, 409, 411, 412, 413, 52, 712, 714
 García, José A. 880
 García, Jorge B. 1043
 Garcia, Lynne S. 762
 Garcia, Maria P 792, 109, 116

Important Note: The number(s) following author names refers to the abstract number.

A-7

- Garcia Zattera, MJ 855
 Garcia-Miss, Maria R. 507
 García-Rivera, Enid J. 468
 Gardella, Catalina E. 114
 Gardner, Ian A. 383
 Gargallo, Domingo 530, 864
 Gargallo-Viola, Domingo 1004, 169, 528, 529, 535
 Garman, Gabriel W. 121
 Garner, Paul 67
 Garrison, Aura 957
 Garry, Robert 277
 Garuti, Helena 528
 Gasasira, Anne F. 320
 Gasparetto, Chiara 1036
 Gatarayiha, Jean Philip 874
 Gatei, Wangeci 625, 945
 Gattton, Michelle 358
 Gautam, Anirudh 867
 Gauthier, C line 862
 Gavidia, Cesar M. 714, 388, 410
 Gaye, Oumar 306
 Gbotosho, G.o. 857
 Gbotosho, Grace O. 187, 858
 Gbotosho, Shola 429
 Gebregeorgis, Elizabeth 220
 Gebru, Solomon 504
 Geisbert, Joan 660
 Geisbert, Thomas W. 660
 Gelbart, William M. 234, 931
 Genton, Blaise 33, 58
 Gerade, Benjamin 726
 Gerard, Craig 839, 971
 Gerard, Vital 69
 Gerena, Lucia 335, 869, 871, 873, 878
 Gettayacamin, Montip 335, 538, 984
 Ghai, Mala 215
 Ghanaie, Roxana M. 71
 Ghani, Azra 1103
 Ghansah, Anita 211
 Gheba, Danial 695
 Ghebremeskel, Tewolde 434
 Gholamreza, Hatam 261
 Ghosh, Anil 667
 Giaya, Kris 800
 Gibbons, Robert V. 445, 466, 472, 803, 1010, 370
 Gibbs, E P. 815
 Giersing, Birgitte 1096
 Gilchrist, Carol A. 384, 619
 Gill, Christopher J. 432
 Gill, Sarjeet 806
 Gilles, Jean-Elie 69
 Gillespie, Joseph J. 40
 Gilman, Robert 381, 406, 410, 568, 624, 914, 945
 Gilman, Robert H. 1026, 1082, 1083, 388, 389, 391, 392, 393, 412, 52, 712, 714, 948
 Gimite, Dereje D. 504
 Gingrich, John B. 253
 Girard, Yvette A. 809, 120
 Giraudoux, Patrick 711, 715
 Girerd, Yves 458
 Giriputro, Sardikin 1088
 Giron, Jessica M. 138
 Gir n, Mar a 776, 778, 779
 Girouard, Autumn 382
 Giroux, Piera 604
 Gissot, Mathieu 294
 Gitawati, R 159, 339, 995
 Githeko, Andrew K. 1057, 609
 Githure, John I. 615, 616
 Gittleman, John L. 955
 Glaser, Amy L. 940
 Glass, Gregory E. 677, 80
 Glen, Jacqueline J. J. 578
 Gnerre, Sante 326
 Goba, Augustin 277
 Gobert, Geoffrey N. 953
 Goddard, Katrina A. B. 1009
 Goel, Priyanka 1019, 1021
 Goethert, Heidi K. 360
 Golay, Xavier 992
 Goldberg, Tony 932
 Golding, Marina 517
 Goldman, Emily L. 252
 Goldman, Ira 190
 Goldmann, Donald A. 48
 Goldstein, Carlos 438
 Gollob, Kenneth J. 451
 Gomes, Carlos E. M. 1061
 Gomez, Ana 1031
 G mez, Christian 143
 Gomez, Gloria P. 390
 Gomez, Tania 823
 G mez, Vanesa 528, 529, 530
 G mez de las Heras, Federico 1004, 169
 G mez-Benavides, Jorge 642
 G mez-de-las Heras, Federico 530
 Gomez-Machorro, Consuelo 612
 Goncalves, Edson 1028
 Gong, Hong-Fei 243
 Gonzaga, Victor 73
 Gonzaga, V 92
 Gonzales, Anthony 47
 Gonzales, Armando E. 389, 390, 391, 392, 712
 Gonzales, Ryan O. 631
 Gonzalez, Alcides 367
 Gonzalez, Armando 410
 Gonzalez, Anthony 701
 Gonzalez, Armando E. 388, 409, 413, 52, 714
 Gonzalez, Brunnell 499
 Gonz lez, Daniel 101
 Gonz lez, Gloria 823
 Gonzalez, Letty 885
 Gonzalez, Rodrigo J. 347
 Gonz lez De la Rosa, Claudia H. 259
 Gonzalez-Ceron, Lilia 1076
 Gonzalez-Guzman, Rosa 54
 Gonzalez-Ramirez, Claudia 836
 Gonzalez, Guillermo 392, 393
 Good, Michael 358
 Gorchakov, Rodion 640
 Gordeuk, Victor R. 853
 Gordon, Emily 605
 Gottschalk, Marcelo 499
 Gotuzzo, Eduardo 1107, 735
 Goudsmit, Jaap 1051
 Gould, Ernest A. 369
 Govea- Arregu n, Arturo 144
 Gowda, A. S. Prakasha 511
 Gowda, D. Channe 511, 512, 570
 Gowda, Kalpana 1050, 899
 Gowdahalli, Krishnegowda 512
 Grabowsky, Mark 692
 Gracia, Fernando 958
 Graczyk, Thaddeus K. 382, 450, 80
 Grady, Katharine K. 190, 534
 Grais, Rebecca F. 661
 Gramaglia, Irene 209
 Granger, D 159, 339
 Granger, Kelly 50
 Grant, Dorsey 373
 Green, Emily N. G. 1042
 Green, Karen 798
 Green, Michael 311
 Green, Sharone 1010, 1044
 Green, S 370
 Greene, Jennifer 562
 Greenhouse, Bryan 673
 Greenwald, David L. 457
 Greenwood, Brian M. 988
 Gregory, Robin 170
 Gregson, Aric 1101
 Grenfell, Bryan T. 661
 Griffin, Carrie E. 189
 Griffith, Matthew E. 405
 Griffiths, Jeffrey K. 771, 959
 Griffiths, Michael 1008
 Grillet, Guillaume 1033
 Grills, Ardath 215
 Grimberg, Brian 1102, 155, 514
 Grinstein, Sergio 852
 Grisolia, Antonella 1036, 431, 759, 760
 Griswold, Marcus W. 236
 Gritsun, Tamara S. 369
 Grob, Natalia 705
 Grolla, Allen 660
 Gros, Philippe 160, 162, 166
 Gross, Diane 50
 Grosskurth, Heiner 325
 Gruener, Beate 751
 Grushko, Olga 1017
 Gu, Jun 1097
 Gu, Xueguang 1073
 Gu, Zhiping 891
 Guclu, Zeynep 941
 Guderian, Jeff 500
 Guelbeogo, Wamdaogo M. 229
 Guerin, Philippe J. 661, 700
 Guerrant, Richard L. 136, 266, 399, 496, 619, 782, 789, 943, 944, 949
 Guevara, Carolina 767
 Gugssa, Ayele 504
 Guiguemde, Robert T. 373
 Guill n, Gerardo 106
 Guillen, Genny 828
 Guillen, Ninoshtka 1031
 Guillerm, Martine 419
 Guillailloux, Nadia 90
 Guimaraes, Georgia F. 796
 Guimar es, Luiz H. S. 1035, 57, 1030
 Guindo, Ando B. 200
 Guindo, Ousmane 1100
 Gulia, Sandrine 456, 458
 Gundersen, Svein G. 70
 Gundersen, Thomas E. 70
 G nther, Stephan 664
 Gupta, Lalita 666, 923
 Gupta, M P. 265
 Gurarie, David 1071
 G rter, Ricardo E. 5
 G rtler, Ricardo E. 738, 739
 Gut, Jiri 181
 Guthmann, Jean-Paul 307, 419, 425
 Guthrie, Benjamin L. 133
 Gutierrez, Ger nimo 1043
 Gutierrez, Victoria 109, 116
 Gutteridge, Clare E. 184
 Guttieri, Mary 277
 Guzman, Guadalupe 473
 Guzman, Maria G. 100, 101, 102, 103, 106, 107, 108, 469, 99
 Guzman Tirado, Maria G. 369
 Gwadz, Robert 923
 Gwer, Samson 342
 Gyan, Ben A. 574, 890
 Gyasi, Richard K. 851
 Gyorkos, Theresa W. 1104, 386

H

- Habbema, J. Dik F. 350
 Hackett, Laura E. 940
 Hackney, Jason A. 621
 Hadioemarto, Panji F. 467
 Haerberle, Adam S. 61, 178, 338
 Hafi, Zen 420, 467
 Hahlen, Bernard 300
 Hahn, Matthew W. 1014, 18
 Haidara, Fadima C. 1084, 1085, 698, 757
 Haiti Research Team, Gonaives 813
 Hajjeh, Rana 60
 Halbur, Patrick G. 1041
 Haldar, Kasturi 515
 Hall, Chris A. 503, 835

A-8

Important Note: The number(s) following author names refers to the abstract number.

- Hall, Eric R. 406
Hallamore, Sandra 357
Halpaus, J. 599
Halstead, Scott B. 369, 99, 100, 469
Halvorsen, Bente L. 70
Hamano, Shinjiro 384
Hamburger, Joseph 627, 736
Hamer, Davidson H. 31, 423, 86, 771, 85
Hamer, Gabe 932
Hamer, Gabel L. 938
Hammond, Martin 234, 931
Hammond, Samantha N. 1094, 367, 368
Han, Chao 864
Han, Eun-Taek 1047, 1048, 532
Hanafi, Hanafi A. 65, 7, 91
Hancock, Kathy 409, 52
Handali, Sukwan 409, 413, 52
Handfield, Martin 1067
Hanif, Beenish 48
Hannah, Michele F. 677
Hansen, Gail 278
Hansen, Immo A. 909
Hanson, Chris 615
Hanson, Kara 434
Hanson, Todd 121
Hansukjariya, Pranee 538
Hanwisai, Suthatta 87
Happi, Christian T. 187, 858, 429
Happi, Tiencha C. 722, 857
Hapuarachchi, Hapuarachchige C. 551
Haque, Rashidul 380, 622
Harajli, Habeeb 150
Haralambou, George 745
Harb, Omar S. 647
Harbach, Ralph E. 908
Harker, Brent 612
Harkins, Timothy 891
Harrell, Andrew 182
Harrington, Laura C. 243, 603, 726
Harris, Eva 1092, 1093, 1094, 111, 367, 368
Harris, Ivor 171
Harris, Jason B. 290
Harris, Lazenias 50
Harrison, Bruce A. 602
Harrison, Travis 515
Hart, C.A. 625
Härter, Georg 713
Hartl, Daniel L. 326, 328
Hartmann, Katherine E. 32
Hartskeerl, Rudy 101
Hartwig, Carmony L. 652
Harun, Syahrial 439
Harvey, Jeffrey 493
Harvey, William R. 15
Hasan, Rumina 48
Haseeb, M. A. 271
Hashimoto, Caryn 216
Hashmi, sarwar 708
Hassan, Hassan K. 605, 611
Hassan, Naureh 837
Hassoun, Ameer 750
Hassoun, Ali 750
Hatfull, Graham F. 327
Hatta, Mochammad 1064
Hauer, M. Claire 19
Havenga, Menzo 1051
Havlikova, Zuzana 428
Havliř, Diane 320
Hawel, Leo 650
Hawkins, Eddy 509
Hawkins, Vivian N. 544
Hawley, Joshua S. 405
Hayati, Nur 467
Hayes, Curtis G. 457
Haynes, Pat 278
Hayton, Karen 295
Hazelton, Paul 665
Hazlett, Fred E. 346
He, Junkun 273, 274
He, Yuxian 1024
Heady, Tiffany N. 335, 869
Heiss, Rebecca S. 940
Helmy, Hanan 480
Hemme, Ryan R. 246
Hemphill, Andrew 618
Hencke, Janice D. 762
Hendrix, Rose 891
Heppner, Christian T. 218
Heppner, Gray 892
Herbein, Joel F. 762
Hernan, Aurora 436
Hernandez, Jorge 785
Hernandez, Jean N. 886
Hernández, Nelly 776, 778, 779
Hernández- Nava, Gustavo A. 113
Herrera, Beatriz 1083
Herrera, Efrén Dionisio 555
Herrera, Flor 862
Herrera, Patricia 735
Herrera-Najera, Carla 508
Herreros, Esperanza 1004, 169, 535
Herricks, Thurston 163
Herwaldt, Barbara L. 141, 1037
Hess, Brian 836
Hetzl, Manuel W. 29
Hewison, Cathy 1033
Hibberd, Martin L. 1007
Hickman, Merrit 846
Hidalgo, Marylin 785
Hien, Tran Tinh 1008, 1109
Higazi, Tarig B. 293
Higgins, LeeAnn 21
Higgs, Stephen 120, 806, 809, 924
Hightower, Alan 969
Hill, Adrian 517
Hill, Catherine A. 1
Hill, Vincent R. 758
Hillier, Stephen D. 553
Hillman, Jeffrey 1067
Hinrichs, David 337, 336, 870
Hira, Parsotam R. 56
Hirano, Eduardo W. 313
Hirayama, K 465
Hirt, Deborah 516
Hittner, James B. 207, 208, 560
Hiwat, Helene 244
Hixon, Mark S. 122
Hjelle, Brian 679
Hoang, Dang Minh 1008
Hoang, Dao N. 465
Hoang, Long T. 1007
Hochberg, Lisa 416
Hodgson, Abraham V. O. 211, 449, 559, 890
Hoel, David F. 38, 65, 91, 7
Hoffman, Marshall M. 184
Hoffman, Stephen L. 449, 559
Hoffmann, Erika E. H. E. 196
Hogan, Joseph 1070
Hokke, Cornelis H. 732
Holbrook, Michael R. 983
Holeckova, Katarina 428
Holman, M. 599
Holman, Robert 278
Holmes, Edward C. 99
Hong, Sung-Jong 270
Hong, Sung-Tae 790
Hong, Young 1017
Hongsri-muang, Thongchai 863
Hook, Christa 419
Hooper, Jay W. 284
Hooshmand, B 1032
Hopkins, Donal R 90
Hopkins, Donald R. 1069
Hopkins, Heidi 305, 672, 721
Hopkins, John M. 848
Hospenthal, Duane R. 405
Hotez, Peter 1103, 823
Hotma, L 719
Hottel, Hannah 957
Houghton, Raymond L. 500
Houng, Huo-Shu H. 801
Haupt, Eric 384
House, Brent L. 221
Hovette, Philippe 977
Howell, James 278
Howell, Paul I. 251, 586
Hsiao, Christine Chia-Hung 838
Hu, Jinhong 1097
Hu, Rong 651
Hu, Renjie 701
Huaman, Alfredo 767
Huaman, A. 92
Huaman, Maria Cecilia 1099
Huang, Fengying 1048
Huang, Juan 600, 604
Huang, Jyh-Hsiung 808
Huang, Shuhui 220
Huang, Xiudong 1096, 217
Huang, Yuefang 486
Huang, Yan-Jang S. 808
Huarcaya, Erick F. 1066
Hubbard, Alan 1073
Hubbard, Gene B. 834
Huber, Curtis S. 534
Huddler, Donald P. 869
Hudson, Peter J. 477
Hudson, Thomas H. 869
Hugger, Erin 864
Hughes, Mark T. 643
Huhn, Gregory 278
Huo, Bernadette P. John. 1054
Hui, George 216
Hume, Jen 913
Humphreys, Tom D. 983
Hunsperger, Elizabeth A. 802, 813
Hunt, Donald F. 21
Hunter, Robert 1038
Husch, Alfred 1087
Hutchinson, M. 599
Hutchinson, Michael 928
Hutson, Christina L. 680
Hviid, Lars 889, 973
-
- Iakovou, Christos 744
Iakovou, Chris 745
Iannaccone, Geno 332
Ibadova, Gulnara A. 286, 314
Ibarra de Palacios, Patricia 755
Ibrahim, B 1069
Ichimori, Kazuyo 345
Idro, Richard 342
Igietseme, Joseph U. 149
Iheanacho, Chinwe E. 272
Ijams, Paul 36
Ilika, Amobi L. 84
Imade, Godwin 564
Imani, Reza A. 628
Imming, Paul 761
Impoinvil, Daniel E. 615
In Peru, For the Cysticercosis Working Group 389, 390, 391, 392, 393, 411, 412, 712
Incardona, Sandra 550
Insuan, Sucheera 421
Iqbal, Jamshaid 56
Iriemenam, Nnaemeka 206
Iriko, Hideyuki 1047, 1048
Ishengoma, Deus 448, 483
Ismail, Tharwat 60
Israelski, Dennis M. 493
Issa, Nebie 197
Issiaka, Soulama 197
Iya, Daniel 564
Iyer, Santhi 47
Izquierdo, Alieny 106

- J**
- Jabbar, Adnan A. 742
 Jacob, Benjamin G. 255
 Jacobs, William R. 327
 Jacobs-Lorena, Marcelo 582, 667
 Jaffe, David 326
 Jain, Seema 1086
 Jain, Vidhan 991
 Jambou, Ronan 205, 210, 30, 355, 655, 977
 James, Anthony A. 225
 James, Calvin B. L. 257
 Janda, Kim D. 122, 819
 Jani, Dewal 332
 Jansen, FH 334, 542, 543, 855
 Jansen, Frans H. 531
 Jansen-Luts, Annemie 543
 Janusz, Aimee 701
 Jaramillo-Gutierrez, Giovanna 596, 923
 Jarilla, Blanca 24
 Jarman, Richard G. 1010, 370, 466, 472, 803
 Jasinskiene, Nijole 225
 Javed, Anam 20
 Jawara, Musa 231
 Jean Bosco, Ouedraogo 192
 Jean-François, Vély 90
 Jedlicka, Anne E. 677
 Jedrzejewski, S. 382
 Jensen, Anne 170
 Jensen, Anja T. R. 172
 Jeri, César 568
 Jeronimo, Selma M. B. 685, 1061
 Ji, Zhuo 790
 Jia, Xainli 668
 Jia, Yongqing 478, 812
 Jiang, Daojun 704
 Jiang, George 204, 903
 Jiang, Hongying 548
 Jiang, Jianlin 1049, 301
 Jiang, Ju 361, 365
 Jiang, Lubin 157
 Jiang, Shibo 1024
 Jilma, Bernd 985
 Jimenez, Cristina 269
 Jiménez, Elena 1004
 Jimenez, Juan A. 389, 390, 391, 392, 411
 Jimenez, Matilde 366
 Jiménez, Magdalena 529
 Jiménez-Díaz, Belén 530
 Jiménez-Díaz, M^a Belén 1004, 528, 529, 535
 Jin, Ling 1047, 1048
 Jin, Xia 105, 1095
 Jinadu, Munirah Y. 1069
 Jirage, Dayadevi 873
 Jituboh, David 1081
 Jiz, Mario A. 24
 Joa, David 90
 Joel, Pradeep K. 991
- John, Bernadette 1055
 John, Chandy C. 34, 520, 883, 888, 895
 Johnson, Cynthia 1105
 Johnson, Jacob 174
 Johnson, Joey 364
 Johnson, J. 599
 Johnson, Jacob D. 871
 Johnson, Stephanie T. 264
 Johnson, Todd 804
 Johnston, Stephanie P. 433, 758, 1037, 312
 Joloba, Moses 375
 Jones, Franca 791
 Jones, Frances M. 26
 Jones, Franca R. 138, 46
 Jones, Jeffrey L. 89
 Jones, J M. 370
 Jones, James W. 245, 256, 726
 Jones, Kelly 620
 Jones, Kate 955
 Jones, Matthew J. 477
 Jones, Malcolm K. 711
 Jones, Matthew L. 841
 Jones, Steven 660
 Jones, Tim F. 936
 Jong, Elaine 985
 Jongsakul, Krisada 374, 87
 Jongwutiwes, Somchai 863, 898
 Jonsson, Colleen B. 966, 964
 Jönsson, Maria 656
 Jordan, Stephen J. 900
 Jornrakate, Possawat 49, 699
 Joseph, Lawrence 631
 Joseph, Serene A. 1104
 Joshi, Uday B. 692
 Joubert, Ava 1105
 Joy, Deirdre A. 1076
 Judy, Barbara 637
 Jukes, Matthew C. H. 422
 Juliano, Jonathan J. 12
 Juliano, Steven A. 236
 Jullu, Boniphace S. 319
 Juncansen, Camila 196
 Juompan, Laure 1048
 Justino, John 434
 Justo, Carlos 823
 Jusuf, Hadi 1088
- K**
- K'ogal, Amos 344
 Ka, Babacar 977
 Kabarisa, Tharcisse 874
 Kabatereine, Narcis B. 26, 1068
 Kabir, Mazbahul 500
 Kabir, Mamun 622
 Kabir, M J. 1032
 Kabra, Sushil K. 288
 Kachapati, Kritika 577
 Kachur, S. Patrick 671, 10, 1075, 670
- KadduMukasa, Mark 654
 Kadhiraivan, Tamilarasu 288
 Kadyrov, Akbar 961, 963
 Kaewpan, Anek 699
 Kafatos, Fotis C. 234, 931
 Kager, Piet A. 497
 Kahindi, Samuel 398
 Kain, Kevin C. 162, 166, 567, 852, 893, 975, 160
 Kaiser, Karine 1003
 Kal, Alphonsus 1069
 Kalavsky, Erich 428
 Kaleebu, Pontiano 325
 Kalilani, Linda V. 658, 414
 Kalinda, Alvin S. 526
 Kaliraj, Perumal 1025
 Kalsy, Anuj 291
 Kalugina, Lyudmila 963
 Kalume, Dario E. 667
 Kalyanasundaram, Ramaswamy 1022, 25
 Kamate, Beh 1100
 Kambale, Wilson 672
 Kamden, Colince 589
 Kamhawi, Shaden 741
 Kamiza, Steve 994
 Kamugisha, Erasmus 654
 Kamwati, S.K 625
 Kamwendo, Deborah 323
 Kamy, Moses R. 305, 320, 343, 672, 717, 896, 1000, 552
 Kanagalem, E 719
 Kande Betu-Ku-Mesu, Victor 145
 Kanefsky, Jeannette 22
 Kaneko, Osamu 1047
 Kang, Shin-Yong 270
 Kania, Stephen 964
 Kante, Ousmane 856
 Kanzok, Stefan M. 667
 Kao, Chuan-Liang 808
 Kapan, Durrell D. 401
 Kapil, Arti 288
 Kaplan, Jenifer 80
 Kaplan, Ray M. 130
 Kapoor, Anil 441, 781
 Karanja, Diana 969
 Karem, Kevin 278, 680
 Karema, Corine 875
 Karen, Kevin 636
 Kariuki, Curtis 627, 736
 Kariuki, Michael 572
 Kariuki, Simon 203, 300
 Kariuki, Thomas M. 734
 Karunajeewa, Harin 993
 Karunarate, Parakma S. H. 17
 Karunaweera, Nadira D. 328
 Kasehagen, Laurin J. 33
 Kasper, Jacob M. 214
 Kastens, Will 1034, 33, 346
 Kasvosve, Ishmael 853
 Katabarwa, Moses N. 1069, 78
 Kataraihya, Johannes 448
 Kato, Elizabeth 884
- Katsnelson, Svetlana 993
 Katunguka-Rwakishaya, Eli 26
 Katz, Jackie 1088
 Katz, Jacqueline M. 756
 Katz, Kevin C. 893
 Katz, Mark A. 756
 Kaul, Surinder 131
 Kauth, Christian 203
 Kawai, B. 292
 Kawai, Vivian 1026
 Kayondo, Jonathan K. 232
 Kazura, James W. 1034, 33, 346, 377, 882, 895, 562
 Keating, Joseph 615
 Kebaier, Chahnaz 579
 Keebler, jon 299
 Keen, Jessica 975
 Keene, Kimberly M. 960
 Keene, Kim M. 965
 Keiser, Tracy 971
 Keita, Adama D. 125
 Keita, Moussa 1078
 Keita, Mahamadou M. 1084
 Keita, Mamadou M. 1084
 Keita, Mahamadou M. 1085
 Keita, Mamadou M. 1085, 698
 Keita, Mahamadou M. 698, 753, 757
 Keita, Mamadou M. 757
 Keita, Somita 502
 Keith, James H. 1089
 Kekitiinwa, Adeodata 320
 Kellar, Kathryn L. 450
 Keller, Christopher C. 156, 158, 207, 208, 352, 491, 560, 997
 Keller, Thomas 564
 Kelly, Greg 357
 Kelly, Jane X. 870, 872, 336, 337, 539, 540
 Kelly-Hope, Louise A. 313, 43
 Kenangalem, E 159, 339, 995
 Kengluocha, Ampornpan 245
 Kennedy, Jeffrey S. 1044
 Kent, Kim A. 814
 Kent, Rebekah J. 1060, 241
 Kern, Florian 108
 Kern, Marcia 1017
 Kern, Peter 713, 751
 Kesavaraju, Banu 236
 Ketloy, Chutitorn 218
 Khabiri, Alireza 837
 Khairnar, Krishna S. 262
 Khali, Diomand 194
 Khalid, Nabila 56
 Khalil, Rayane 845
 Khan, Imtiaz 131, 742
 Khan, Salwa 380
 Khan, Tariq A. 745
 Khasakhala, Lincoln 422
 Kheng, Sim 547
 Khihembo, Macklyn 553
 Khim, Nimol 550
 Khodiev, Aybek V. 286, 314

A-10

Important Note: The number(s) following author names refers to the abstract number.

- Khodjaev, Shabot 961
 Khoka, Miriam 88
 Khouma, Mbaye 434
 Khuntirat, Benjawan 532
 Khyuziahmetova, Ilseyar 963
 Kiang, Richard K. 1079
 Kieffer, Mary Pat 321
 Kiguli, James 419
 Kijchalao, Udom 245, 256
 Kikuchi, M 465
 Killeen, Gerry 1054, 1055
 Kilpatrick, A. Marm 477
 Kim, Andrea 437
 Kim, Andrea A. 312
 Kim, Chang-Gyun 153
 Kim, Hye-Sun 232
 Kim, Jung Yeon 258
 Kim, Kami 294
 Kim, Tong Soo 258
 Kim, Tae Im 270
 Kim, Young-A 153
 Kines, Kristine J. 953
 King, Christopher 1034
 King, Chwan-Chuen 808
 King, Charles H. 1072, 627, 662, 736, 737, 951
 King, Christopher L. 1102, 509, 668, 987
 King, C. R. 1050, 899
 King, Jonathan 345
 King, Richter C. 577
 Kiniboro, Benson 33
 Kinyua, Antony 1105
 Kirby, Paula 180
 Kirby-Allen, Melanie 160, 166
 Kirkman, Laura 978
 Kirkpatrick, Beth D. 380
 Kironde, Fred 654
 Kiszewski, A. 599
 Kitchener, Scott 170
 Kitron, Uriel D. 938, 5, 737, 738, 739, 932
 Kitthawee, Sangvorn 726
 Kivihya-Ndugga, Lydia E. A. 703
 Kizito, Dennison 325
 Kizza, Moses 553
 Kjos, Sonia A. 40
 Klade, Christoph 984, 985
 Klatser, Paul R. 1064, 403, 703
 Klausner, Jeffrey D. 46
 Klee, Lauren M. 361
 Klei, Thomas R. 710, 818
 Klein, Robert E. 347
 Klein, Sabra L. 677, 678, 80
 Kleinschmidt, Immo 1081, 9
 Klena, John D. 289, 65
 Kleschenko, Yuliya 330, 686, 688, 689
 Klimczak, Leszek J. 707
 Klimov, Alexander X. 1088, 756
 Kline, Daniel L. 38, 248
 Klingler, Anton 985
 Klion, Amy D. 1023, 125, 351, 821
 Klungthong, Chonticha 371, 370
 Knols, Bart G. J. 1055, 222, 919, 1054
 Knox, Jessica 909
 Knuchel, Marlyse C. 319
 Ko, Albert 81
 Kob-Asa, Teerayot 863
 Kobbe, Robin 309
 Kochel, Tadeusz J. 457, 767, 804
 Koebele, Carey 923
 Koehler, Theresa 1067
 Koekoemer, Lizette L. 584
 Koenraadt, Constantianus J. M. 243, 245, 256, 603
 Koenraadt, S 370
 Koita, Ousmane A. 165, 304, 331, 866
 Kokoskin, Evelyne 386
 Kokwaro, Gilbert 720
 Kolb, Carol 556
 Kolk, Arend J. H. 703
 Kollaritsch, Herwig 985
 Komar, Nicholas 280, 281, 282
 Komatsu, Natália T. 196
 Kombila, Maryvonne 329
 Komilov, Nemat 961, 963
 Konare, Sekou 1078
 Konate, Lassana 231
 Konate, Siaka 484
 Konda, Kelika A. 138, 46
 Kondig, John P. 957
 Kone, Aminatou 379
 Kone, Abdoulaye K. 200, 892
 Kone, Mamady 856
 Koneru, Bhanumati 797
 Kongkasuriyachai, Darin 861
 Konovalova, Svetlana 577
 Konradsen, Flemming 551
 Koram, Kwadwo 449
 Koram, Kojo 559
 Koram, Kwadwo A. 199, 211, 574, 890, 65
 Korinek, Maria 985
 Korir, Cindy 521, 840, 845
 Kort, Jens J. 47
 Korten, Simone 817
 Koru, Ozgur 941
 Kosar, Aaron J. 536
 Kosasih, Herman 1088, 420, 467
 Kosek, Margaret 1083
 Kosoy, Michael 80
 Koster, Frederik 958
 Kotloff, Karen L. 1084, 1085, 698, 753, 757
 Kou, Zhihua 1095
 Koukounari, Artemis 1068
 Kouri, Gustavo 100, 108, 469
 Kouri Flores, Gustavo 369, 99
 Kovac, Paul 291
 Kovalenko, Victor A. 52
 Kozyr, Natalia 847, 891, 972
 Kraemer, Susan M. 296
 Kraemer, Sue M. 327
 Krafur, Elliot S. 359
 Kramer, Laura D. 1040, 478, 730, 812, 477
 Krasaesub, Somporn 421
 Krastins, Bryan 290
 Kraus, Annette A. 110
 Krause, Darren 358
 Krause, Peter 832
 Krcmery, Vladimir 428
 Krebs, John W. 402
 Kreishman-Deitrick, Mara 869
 Kreamsner, Peter G. 1101
 Kreuels, Benno 309
 Kreuzberg, Christina 309
 Krithika, K N. 1025
 Kroeger, Axel 614
 Krogstad, Donald J. 165, 304, 331, 866, 868
 Kroon, Erna 636
 Krudsood, Srivicha 992
 Krungthong, Chonticha 472, 803
 Kruszon-Moran, Deanna 89
 Krzych, Urszula 1048
 Krzywinski, Jaroslaw 926
 Kuaha, Kannika 183
 Kuan, Guillermina M. 367
 Kubak, Bernard 1038
 Kubofcik, Joseph 1019, 1021, 707, 821
 Kucerova, Zuzana 149, 947
 Kuddo, Thea 853
 Kudiabor, Emmanuel 956
 Kuehnert, Matthew 1038
 Kuijper, Sjoukje 703
 Kuk, Salih 353
 Kum-Arb, Utaiwan 218, 566
 Kumar, Sanjai 332
 Kumar, Sumit 519
 Kumar, Sanjeev 596, 666, 923
 Kumazaki, Kaori 563
 Kumemura, Masashi 182
 Kumi, Winifred O. 407
 Kun, Heather 1038
 Kung'u, Amos 215
 Kuniholm, Mark H. 659
 Kunkel, Kenneth E. 938
 Kurane, Ichiro 474
 Kurewa, Nyaradzai E. 137
 Kurniawan, Agnes 946
 Kurosu, Takeshi 464
 Kurtis, Jonathan 28
 Kurtis, Jonathan D. 1070, 22, 23, 24, 417
 Kusi, Kwadwo A. 574, 890
 Kuwuor, Dickens 883
 Kweku, M. 65
 Kwenang, Agnes 1064
 Kwiek, Jesse J. 658, 12
 Kwitshana, Zilungile L. 1106
 Kyabayinze, Daniel J. 321
 Kyes, Sue 296
 Kyle, Dennis 174, 538
 Kyle, Jennifer L. 1092, 1093
 Kyosiimire-Lugemwa, Jacqueline 325

L

- LaBeaud, A. Desiree 662
 Laboonchai, Anintita 421
 Lacerda, Roberta M. 1061
 Laclette, Juan Pedro 53
 LaCrue, Alexis N. 572
 LaFleur, Gayelyn 209
 Lagos, Rosanna 283
 Laguna-Torres, Alberto 767
 Laguna-Torres, Victor A. 133
 Lahlou, Rita M. 622
 Lahuerta, Maria 324
 Lahad, Ferdinand J. 1079
 Lal, Chandra S. Lal. 446
 Lam, My T. 1005
 Lama, Javier R. 133
 Lammie, Patrick L. 130, 345, 349
 Lampah, DA 159, 339, 995
 Lampman, R. 599
 Lamptey, Helena 890
 Lander, Eric 326
 Landis, Sarah H. 32
 Lane, Kristin D. 657
 Laney, Sandra J. 483
 Lang, Jean 458
 Langdon, Gretchen C. 22, 23, 24, 28
 Langefeld, Iris 309
 Langevin, Stanley A. 1042
 Lanteri, Charlotte A. 335
 Lanzaro, Gregory 397, 591, 594
 Laothamatas, Jiraporn 992
 Lapp, Stacey A. 302, 521
 Lappin, Michael R. 383
 LaRocque, Regina C. 290
 Larsen, Christian 891
 Larsen, Tom 284
 Larsson, Cathy 380
 Latour, Christine 1003, 876
 Laufer, Miriam K. 884
 Lautze, Sue 959
 Lavenberg, Glenn 777
 Lawrence, Gena 1038
 Lawson, Alexander M. 669
 Lawson, Benard W. L. 1057
 Lawson, Daniel O. 931, 234
 Lawyer, Phillip 741
 Lazarus, Wilfred 123
 Le, Bao T. 1005
 Le, Lien B. 1005
 Le, Minh Vien T. 287
 Le, Thuy T. 1005
 Le Bras, Jacques 297
 Le Roch, Karine G. 303
 Leach, Amanda 892
 LeBreton, Matthew 659
 Lederman, Edith R. 191

- Lee, Chang-Chun 808
 Lee, Clarence M. 504
 Lee, Eng-Hong 573
 Lee, Hyo-Il 153
 Lee, Hyeong Woo 258
 Lee, Hyejon 43
 Lee, Norman N. 227
 Lee, Patricia 869
 Lee, Soon-Hyung 790
 Lee, Young Hee 258
 Lee, Yeuk-Mui 409, 52
 Lee, Yoosook 594
 Leenstra, Tjalling 1070, 23, 28, 417, 497
 Leger, Paul 136, 496
 Legrand, Anne-Marie 485
 Legros, Dominique 425
 Lehmann, Tovi 587, 724, 913, 923, 926
 Lehrer, Axel T. 983, 980
 Leiby, David A. 264, 500, 1038
 Leitner, Wolfgang 1105
 Lekana-Douki, Jean Bernard 329
 Lekprasert, Varinee 992
 Leland, M. Michelle 834
 Lemnge, Martha M. 448
 Lengeler, Christian 29, 434, 8, 887
 Lenhart, Audrey 614, 693
 Lenta, Bruno N. 181
 Leo, Megan 622
 Leon, Segundo R. 46
 Leowattana, Wattana 992
 Lerdthusnee, Kriangkrai 421
 Lesaffre, E 855
 Lescano, Andrés G. 275, 46, 73, 92, 138
 Levin, Emily 296
 Levin, Mike 342
 Levin, Michael L. 362
 Levine, Gail L. 1050
 Levine, Min 409
 Levine, Myron M. 1084, 1085, 283, 698, 753, 757
 Levis, Silvana 1043, 644
 Levy, Karen 400
 Levy, Michael 614
 Levy, Marc 955
 Levy, Michael Z. 1026
 Lewallen, Susan 340
 Lewis, Sarah 1109
 Lewis, Shawn 964
 Lewockzo, Kenneth 936
 Li, Benwen 704
 Li, Ben-Wen 816
 Li, Cuixia 217
 Li, Cong 238
 Li, Guangyu 119
 Li, Guangfu 512, 570
 Li, Jun 21
 Li, Jinlong 850
 Li, Peter 891
 Li, Qigui 167, 174, 178, 179, 61
 Li, Song 1015
 Li, Shizhu 226
 Li, Shunyu 270
 Li, Song 583
 Li, Wen 705
 Li, Xiaoming 567
 Li, Xinyi 571
 Li, Yu 636
 Li, Yi-Hsuan 808
 Li, Zhen 1097
 Li, Zhimin 790
 Liang, Jennifer L. 345
 Liang, Song 1073
 Libman, Michael D. 386
 Libraty, D H. 370
 Licht, Monica 913
 Licitra, Johnathan L. 243
 Lieberman, Michael M. 983, 1045, 980
 Liebman, Katherine 539, 540
 Liem, Ho Thanh 1109
 Lilley, Bruce M. 611
 Lim, Pharath 550
 Lima, Aldo A. Moreira. 826, 944, 782, 399
 Lima, Maria F. 330, 686, 688, 689
 Lima, Noelia L. 782
 Lima-Junior, Josué C. 198
 Limbach, Keith 1050, 899
 Limbaso, Samson 633
 Limonta, Rubio M. 69
 Limor, Josef 190
 Limsalakpetch, Amporn 218, 566
 Lin, Nianwei 592
 Lin, Neal 808
 Lin, Yun H. 129
 Lindblade, Kim A. 34, 347
 Linde, Nathaniel 640
 Lindstrom, Stephen E. 756
 Linser, Paul J. 16, 223
 Linthicum, Kenneth J. 254, 248
 Lipner, Ettie M. 351
 Liscum, Kathleen R. 263
 Liskova, Anna 428
 Lister, Nicole 986
 Listyaningsih, Erlin 1088, 439, 467
 Liu, Anna 295
 Liu, Bo 983
 Liu, Dongying 641
 Liu, Jian 1097
 Liu, Jun 567
 Liu, YueMin 632
 Llanos-Cuentas, Alejandro 886
 Lloyd, Jennie M. 988
 Lô, Baidy 663
 Loayza, Manuel 424, 93
 Lobban, Kathleen 1105
 Lobo, Neil F. 1016, 1017, 232, 234
 Lockhart, Lauren 380
 Loftis, Amanda D. 1039
 Loftus, Brendan J. 1016
 Logan, Kathleen S. 40
 Logue, Christopher H. 960
 Logue, Chris H. 965
 Loker, Eric S. 628, 733
 Lokida, Dewi 1088
 Lokomba, Victor 32
 Lomasney, Jon W. 515
 Long, Carole A. 1098, 1099, 1100
 Long, Earl 433
 Long, Maureen T. 815
 Long, Truong Hoang 1008
 Looreesuwan, Sornchai 151, 904, 992
 Lopansri, B 159, 339
 Lopera, Luis 714
 López, Jaime 555
 Lopez, Victor 694
 Lopez-Sanchez, Miriam 869, 871, 873
 Lord, Cynthia C. 921
 Lorenzana, Ivette 473
 Lorry, Kerry 33
 Löscher, Thomas 44
 Loss, Scott R. 938, 932
 Louis, Christos 234, 931
 Lounibos, L P. 934, 236, 459
 Lovchik, Janece M. 981
 Lovegrove, Fiona E. 162
 LoVerde, Philip T. 651
 Lovin, Diane 612
 Lowe, Brett 720
 Lowe, Henry 1105
 Lowe, Michael C. 338
 Lowery, Roy J. 572
 Lozano, Celina 101
 Lozano, Sonia 169, 535
 Lozano-Fuentes, Saul 594, 930
 Lu, Bao 839, 971
 Lu, Lydia 312, 437
 Lu, Ziyue 893
 Lubanga, Rosalind 721
 Lubinsky, Anthony 271
 Luby, Stephen P. 42
 Lucas, Carmen 142, 545, 546, 998
 Lucas, Martin 998
 Lucchi, Naomi W. 354
 Lucey, Daniel 416
 Lujemwa, Myers 305
 Lugo-Yarbu, Ana 139, 140
 Lukes, Yvonne 146
 Luo, JianPing 632
 Lustigman, Sara 1022, 1024, 122, 708
 Lydy, Shari L. 1065
 Lyerly, David 789
 Lyke, Kirsten E. 200, 892
 Lynch, Julia 797, 801
 Lynd, Amy 697
 Lyon, Jeffrey A. 849
 Lyons, Art 984
 Lyons, Rick 1067

M

- M, And Nwagwu 561
 Ma, Yajun 226
 Ma'roef, Chairin N. 1088, 439, 467
 MacCallum, Robert M. 234, 931
 MacDonald, Nicholas J. 578, 578, 846
 Macete, Eusebio 1001
 Mach, Ondrej 312, 437
 Machado, Eleuza R. 487
 Machado, Paulo R. 1030
 Machado, Paulo R. L. 1035
 Machado, Paula R. L. 115, 57
 Machado, Rosangela Z. 498
 Machekano, Rhoderick N. 493
 Machevo, Sonia 1001
 Mackenzie, Charles D. 292, 123
 MacLean, Dick 824
 MacLean, J. Dick 386
 MacLeod, William B. 432
 Madebe, Rashid 448
 Madeline, Brice 458
 Madey, Greg 234, 931
 Madhunapantula, SubbaRao V. 511
 Madison, Marisa N. 689
 Madison, Nia 330, 686, 688
 Maduka, Chinyere 78
 Maffei, Joseph G. 812
 Magalhaes, Maria Cecilia F. 796
 Magalhaes, Tereza 593
 Magalhes, Tereza 918
 Magesa, Stephen 483
 Magill, Alan 546
 Magnussen, Pascal 422
 Maguina, Ciro 1066
 Maguiña, Mirtha 791
 Maguire, Jason D. 191, 377
 Mahajan, Simmi 967
 Mahamadou, I 866
 Mahanty, Siddhartha 1098, 1099
 Maharaj, Payal 1042
 Mahathat, Chaiyawat 421
 Maher, Emad 633
 Mahmoud, Eiman 769
 Maiga, Bakary 206
 Maiga, Mahamadou 1078
 Maio, William 526
 Maiteki, Catherine 717
 Maiteki-Sebuguzi, Catherine 343, 552
 Maitland, Kathryn 342
 Maixenchs, María 1001, 674
 Maizel, Margaret S. 82, 83
 Majewska, Hanna 382
 Majura, Albert 14
 Makanga, Michael 720
 Makemba, Ahmed M. 29
 Makobongo, Morris 846
 Makori, Norbert 182
 Makou, Raufou 676

A-12

Important Note: The number(s) following author names refers to the abstract number.

- Maksoud, Mohamed A. 60
 Malafronte, Rosely S. 196
 Malaivichitnond, Suchinda 863
 Malasit, Prida 466
 Malecela, Ezekiel K. 448
 Malecela, Mwele 123
 Malhotra, Indu 987
 Malia, Jennifer A. 133
 Malila, Aggrey 670, 671
 Malkin, Elissa 1097, 1098, 1099
 Malone, John B. 1028
 Malvy, Denis 718
 Mamadou Marouf, Keita 860
 Mamani, Enrique 109, 116
 Mamman, Aisha 423
 Mamman, A. I. 85
 Mamman, Aisha I. 86
 Mammen, Mammen P. 371, 445, 466, 472, 803, 1010, 370
 Manalo, Daria 22
 Mandel, Eric J. 402
 Mandomando, Inacio 324
 Manfras, Burkhard 713
 Mangochi, Bridget 88
 Manguin, Sylvie 590, 908
 Manirakiza, Alexandre 718
 Mann, Amy 948
 Mann, Barbara J. 384
 Mann, Victoria H. 953
 Mannix, Frank 933
 Manoukis, Nicholas C. 1059
 Mantel, Nathalie 458
 Manuel, Manuarii 485
 Manzanedo, Rafael 389, 390, 391, 410
 Manzi, Fatuma 13
 Mao, Chunhong 583
 Maokola, Werner 35, 764
 Maravilla, Pablo 54
 Marcet, Paula L. 738
 Marcos, Luis A. 735
 Mariam, Sylla 860
 Marie, Jérôme 485
 Mariko, Sidiki 1078
 Mariner, Jeffrey 959
 Marinotti, Osvaldo 225
 Marita, Troye-Blomberg 206
 Marks, Florian 309
 Marovich, Mary A. 1091
 Marques, Ernesto T. A. 796
 Marques Jr., Ernesto T. A. 112
 Marquez, J. Gerardo 359
 Marquis, Benoit 321
 Marra, Peter P. 477
 Marrama, Laurence 977
 Marrama Rakotoarivony, Laurence 30
 Marron, Jennifer A. 981
 Marsh, Kevin 357, 517, 986
 Marshall, Jonathon C. 916
 Marshall, John M. 617
 Martin, Diana L. 147, 148, 505
 Martin, Kara 1102
 Martin, Kathy 800
 Martin, Kurt 998
 Martin, Kara K. 668
 Martin, Laura B. 1098, 1099
 Martin, Sandra 660
 Martínez, Antonio 1004
 Martínez, Adolfo 644
 Martínez, Luz Patricia 555
 Martínez, Rafael 106
 Martinez-Muñoz, Jorge P. 230
 Martino-Catt, Susan 384
 Martins, Daniella R. A. 685
 Martins, Livia C. 196, 644
 Martyak, Timothy 980, 983
 Mascola, Laurene 1038, 47, 701
 Masiye, Felix 527
 Massaga, Julius J. 10, 1075
 Masunge, Japhter 312
 Mathew, Leni 41
 Mathieu, Els 349, 482, 75
 Matlashewski, Greg 142
 Matschiner, Alex 277
 Matsuura, Christine 983
 Mattera, Gabriel 377
 Mauceli, Evan 326
 Mawa, Patrice A. 26, 325
 May, JÄ¼rgen 309
 Mayhew, George F. 120, 228, 239
 Mayokola, Werner 13
 Mazhani, Loeto 312
 Mbae, C. 625
 Mbogo, Charles M. 615, 616
 Mbori-Ngacha, Dorothy 321
 Mboup, Soulyemane 303, 326
 Mbui, Jane 285
 McArdle, James L. 439
 McArthur, Julie H. 981
 McAuliffe, Isabel T. 413
 McCall, Philip J. 693, 614, 4, 697
 McCall, Suzanne 405
 McCallum, Fiona J. 986
 McCarthy, Anne E. 554
 McClellan, Holly 220
 McCollum, Andrea M. 188, 190
 McCune, Sheila 928
 McCutchan, Thomas F. 1076
 McDaniel, Philip 421
 McElroy, Brian D. 69
 McElroy, Kate L. 809, 806, 924
 McEvoy, Peter 998
 McFarland, Deborah 145
 McGarvey, Stephen T. 1070, 23, 24, 317, 417, 631
 McGee, Kate M 299
 McGee, Charles E. 809
 McGlone, Bonnie 422
 McGowan, Kevin J. 940
 McGuigan, Kevin G. 59
 McGuire, David 434
 McHenry, John M. 646
 McMahan-Pratt, Diane 968
 McManus, Donald P. 711
 McNamara, David T. 252, 33, 881
 McNeil, Y 339, 995
 McSurdy-Freed, Jeanelle 864
 McVean, Gilean A. T. 299
 Mead, Danny 364
 Mebrahtu, Tsedal 320
 Mechta, Sarah 91
 Medeiros, Melissa S. 826
 Medeiros Filho, Jose 1061
 Medina, Rafael 499
 Medina, Sarimar 1098
 Medina, Yordanka 103, 107
 Medina-Ramirez, Fernando J. 1090
 Medlin, Carol 556
 Meers, Brad 503, 835
 Megnekou, Rosette 889, 973
 Megy, Karyn 234, 931
 Mehlotra, Rajeev K. 377, 378
 Meijerink, Hinta 350
 Melby, Peter C. 506, 650, 786
 Melek, Bekir H. 868
 Mellencamp, Mark M. 815
 Melli, Ann C. 383
 Melnikov, Valery 144
 Melo, Paulo S. 1009
 Melrose, Wayne 743, 783
 Menacho, Julio 1066
 Mendenhall, Ian H. 920
 Mendez, Juan 763, 765, 766, 768, 830, 831
 Mendlovic, Fela 53
 Menéndez, Clara 1001, 324, 674
 Menge, David M. 888
 Mensah, Nathan 559
 Menu, Frederic 397
 Meola, Mark 730
 Mercado-Curiel, Ricardo F. 1011
 Mercereau-Puijalon, Odile 516, 977
 Meremikwu, Martin M. 67
 Merkle, Marion 713
 Mert, Gurkan 941
 Meshnick, Steven R. 32, 537, 550, 12, 323, 414, 658
 Mesirov, Jill 303
 Metta, Emmy 670, 671
 Meydani, Simin N. 771
 Meyer, Christian G. 309
 Meyer, Esmeralda V. S. 298, 301, 524, 844, 848, 198
 Meyers, Adrienne F. A. 665
 Meza, Graciela 1083
 Meza, Rina 46
 Mezarina, Jorge 791
 Mharakurwa, Sungano 1060, 241, 310
 Miaka Mia Bilenge, Constantin 145
 Miao, Jun 571, 898
 Michael, Edwin 1034
 Michael, Sara E. 849
 Michaels, Marian G. 156
 Michaud, Joshua M. 73
 Michaux, Johan R. 590
 Michelin, Ruel 1105
 Michon, Pascal 155, 509, 881
 Mickelson, Nathan J. 121
 Mija, Lizardo 412
 Miles, Aaron P. 1098
 Millhous, Wilbur 167, 174, 878
 Millhous, Wilbur K. 338, 61
 Militello, Kevin T. 214
 Miller, Ann K. 180, 171
 Miller, James R. 600, 604, 691, 927
 Miller, Kenton S. 281
 Miller, Louis H. 1098, 1100, 157, 846, 1099
 Miller, Melissa A. 383
 Miller, Mark A. 43, 313
 Miller, Melissa A. 838
 Miller, Melissa K. 361
 Miller, R. Scott 338
 Miller, R. S. 421
 Miller, Robert S. 533, 770, 773, 775
 Millet, Pascal 307, 718
 Milligan, Paul 754
 Millogo, Niama 724
 Mills-Robertson, Felix 407
 Milner, Dan 326, 557
 Milner, Danny A. 994
 Mion, Geneviève 516
 Milord, Marie Denise 90
 Min, Gi Sik 258
 Min, Michael M. 895
 Min-Oo, Gundula 160, 166
 Minakawa, Noboru 609
 Minch, Kyle 557
 Minh, Truong Tan 1109
 Minnick, Sharon L. 372, 726
 Minor, Katie 1092
 Minta, Daouda 856
 Mintz, Eric D. 1086
 Mirabello, Lisa 915
 Miranda-Verastegui, Cesar 142
 Miri, Emmanuel S. 78, 1069
 Misra, Anoop 288
 Mistry, Anisha 901
 Mitchell, Graham H. 848
 Mitre, Ed 416
 Mitreva, Makedonka 706
 Mitro, Sutrisno 244
 Mittal, Kriti 942
 Mittelstaedt, Brent R. 64
 Mixson, Tonya R. 363
 Mkoji, Gerald M. 733
 Mnalemba, Timothy 557
 Moet, F. J. 1063
 Mofolo, Innocent 414
 Mohamadani, Ahmed A. Mohamadani. 549
 Mohammed, Kakande 553
 Mohandas, Narla 854
 Mohebbali, Mehdi 1032

- Mohran, Zaynab 289
Mojica, Dalis E. 827
Mokolu, Olugbenga A. 85
Mokuolu, Olugbenga A. 86, 31, 423
Molina-cruz, Alvaro 923
Mollinedo, Sergio 488
Molta, Norman 564
Molyneux, Malcolm E. 308, 340, 418, 994
Monagin, Corina 277
Monath, Thomas P. 1044
Moncayo, Abelardo C. 936, 599, 928
Mondal, Dinesh 380, 622
Mone, Tom 1038, 1038
Montalvan, Carmen 545
Montano, Silvia M. 133
Monteiro, Gloria R. 1061
Montenegro, Sílvia M. L. 112
Montes, Martin 1107
Monteville, Marshall R. 289, 633
Montoya, Edinson 412
Moody, Anthony 419
Moody, Erin 936
Moon, James E. 405
Moon, Seung Ung 258
Moonga, Hawela 525, 527, 675
Moore, Amy 280, 281
Moore, Anne C. 145
Moore, Anne M. 1038
Moore, Julie M. 354, 894
Moore, Sarah 608
Moormann, Ann M. 882, 895, 562
Mor, Vincent 1070
Moraes-Ávila, Sandra L. 196
Morales, Adelaida 269
Morales, María A. 1043, 644
Morales, María E. 953
Moran, Manuel 93, 94
Morato, Vanessa 1009
Morcos, Myriam 60
Moreau, Jean Charles 205, 355
Moreira, Luciano A. 582
Moreno, Alberto 1049, 842, 847, 891, 902, 972
Moreno, Elio 139, 140
Moreno, Marta 607
Moreno, Melcenia 828
Mores, Christopher N. 459, 810, 811, 921, 961, 963
Moretz, Samuel 1098
Morgah, Kodjo 482
Morgan, Daniel J. 1030, 1035
Morgan, Juliette 825
Mori, Akio 595
Morier, Luis 100, 102, 103, 106
Morita, K 465
Moro, Pedro L. 752
Morrisey, Joanne 859
Morrison, Amy C. 372, 767, 694
Morrot, Alexandre 1053, 353
Morrow, W J. W. 500
Morsy, Zakaria S. 480
Moser, Melanie A. 433
Mosi, Lydia 396
Moss, Delynn M. 947
Mosser, David M. 989
Mostaafa, Sohair A. 289
Moste, Catherine 456
Moudy, Robin M. 1040
Mouidi, Pacôme 329
Mouline, Karine 1014, 18
Moura, Iaci 450, 453, 758
Moura, Maria L. 1061
Moura, Pedro A. 327, 647
Moyano, Luz M. 392, 393, 411
Moyeed, Rana 614
Mpimbaza, Arthur 341
Mpoudi-Ngole, Eitel 659
Mrisho, Mwifadhi 13, 14
Mshana, Christopher 29
Mshinda, Hassan 13, 14, 29, 319, 35, 764
Msonjela, Yared 448
Mtema, Jephtha 434
Mu, Jianbing 299, 379, 548
Muchai, Samuel 633
Muchiri, Eric M. 1072, 662, 736, 737, 951, 627, 987
Muchohi, Simon 720
Mueller, Dirk 434
Mueller, Ivo 33, 509, 881, 993
Mueller, Joachim 618
Mueller, Kristen 188
Mueller, Norbert 618
Muhangi, Lawrence 325, 553, 26
Muhle, Rebecca A. 327
Mukaka, Mavuto 308
Mukasa, Oscar 35, 764
Mukhopadhyay, Arunima 537
Mukwaya, Louis G. 232
Mulder, Maximilian 406
Mulet, Teresa 1004, 528, 529, 530
Mulinge, E. 625
Mullen, Gregory 1099, 1100
Muller, Ivo 155, 514
Mulligan, Jo 434
Munayco, César V. 568, 642, 792, 424, 93
Mundaca, Carmen C. 424, 93, 94
Munga, Stephen 609
Mungai, Peter L. 1072, 737, 951, 987
Munirathinam, Gnanasekar 1022, 25
Muniz, Pascoal T. 196
Muñoz, Alma 283
Muñoz, Jorge 468
Muñoz, María de Lourdes 230
Muñoz, María d. 114
Munoz, María L. 1011
Munro, James B. 909
Muratova, Olga V. 1098, 667
Murillo, Wendy 473
Murphy, Brian R. 981, 982
Murphy, Sean C. 515
Murray, Clinton K. 405, 421, 60
Murry, Daryl J. 761
Murtada, Ali R. 150
Murugan, Vadivel 1025
Mushi, Adiel 14
Mushinzimana, Emmanuel 609
Musie, Edgar M. 404
Musset, Lise 297
Mut-Martin, Mirza 507
Mutabingwa, Theonest K. 356, 850
Mutebi, Frederick 419
Muth, Sinuon 630
Muwanga, Moses 553
Mwakitalu, Esther 123
Mwanakasale, Victor 432
Mwananyanda, Lawrence 432
Mwapasa, Victor 12
Mwebaza, Norah 1000
Mwinzi, Pauline 969
Myers, Tim 821
Myint, Khin S. 445
Myler, Peter 296
Myrick, Alissa 673, 896
Myrvang, Bjørn 70
Mzayek, Fawaz 331, 868
- ## N
- Nabasumba, Carolyne 419
Nabeth, Pierre 663
Nada, Rania A. 289
Nadal, David 319
Nadim, A 1032
Næss, Lisbeth M. 700
Naficy, Abdullah 574
Nagarkatti, Rana 332
Nagataki, Mitsuru 132
Nagpal, Avinash C. 991
Nahar, Lufton 42
Nahar, Nazmun 42
Naheed, Aliya 702
Nahlen, Bernard 203
Naik, Himanshu 761
Nakata, Yasuto 182
Nakjarung, K 286
Nakkash Chmaissi, Hania N. 150
Namagembe, Allen 721
Namujju, Proscovia B. 26
Nanakorn, Ampon 998
Nanayakkara, Dhammika 865
Naniche, Denise 324
Nantakomon, Duangdao 151
Náquira, Cesar 767
Narayan, Shyam 415, 626
Narayanan, Jothikumar 758
Nardin, Elizabeth H. 1101
Nartey, Helena 890
Narum, David L. 1098, 578, 1099
Nascimento, Eduardo 796
Nascimento, Eliana L. T. 1061
Nascimento, Renata T. 471
Nasveld, Peter 170, 171
Natallia Dziuba, Natallia 637
Natarajan, Jayakumar K. 185
Naumova, Elena 771
Nawaz, Fatima 295
Nayiga, Susan 721
Ndao, Momar 499, 824, 832, 833
Ndayisaba, Gill 874
Nde, Pius 330, 686, 688, 689
Ndeezi, Grace 341
Nderitu, M. 625
Ndiaye, Daouda 303
Ndiaye, Malick 205
Ndiaye, Mamadou 355
Ndibazza, Juliet 26
Ndir, Omar 303
Ndour, Souleymane 30
Neafsey, Dan 326
Ned, Renee 991
Neira, Marco 257
Neira Oviedo, Marco V. 16
Nekhai, Sergei 853
Nelson, Siri 296
Nelson, William M. 457
Nene, Vish 1016
Nene, Vishvanath M. 1
Nettel, Jose A. 1076
Neva, Franklin 824
Neves, Maria F. 498
New, John 964
Newbold, Chris 296
Newhouse, Michael 477
Newire, Enas 60
Newman, Alexandra 278
Newton, Charles R. J. C. 342
Newton, Paul N. 311
Newton- Sánchez, Oscar A. 144, 113
Neyra, Victor 886
Ng'ang'a, Zippora 997
Ng'habi, Kija 1055
Ngoc Rang, Nguyen 1086
Ngouela, Silvère A. 181
Ngoupayo, Joseph 181
Nguyen, Dung 1006
Nguyen, Hung T. 1005
Nguyen, Lam 485
Nguyen, Lan P. T. 465
Nguyen, Minh Dung 1006
Nguyen, Megan L. 264
Nguyen, Thanh Truong 287
Nguyen, Tuan M. 1005
Nguyen, Van Vinh Chau 1006
Nguyen, Vu D. 578
Nguyen Le, My Linh 287
Nguyen Thi, Dung 287
Nguyen-Dinh, Phuc 90
Ni, Haolin 637, 638, 639, 681
Ni, Yi-Sheng 365
Niambele, Mohamed B. 1100

A-14

Important Note: The number(s) following author names refers to the abstract number.

- Niang, Mbayame 663
 Nichol, Stuart T. 665
 Nicholson, William L. 1065, 364
 Nicolay, Nathalie 1033
 Nieto, Nathan C. 96, 98
 Nieto, Prixia 1028
 Nieuwendam, Josta 777
 Nigro, Joseph D. 1079
 Niles, Edward G. 651
 Ninaquispe, Berenice 714
 Nisalak, Ananda 421, 466, 472, 1010, 370
 Nishimune, Yoshitake 464
 Njagi, Kiambo 422
 Njama-Meya, Denise 343, 552, 717
 Njenga, Kaiuki 633
 Njoku, A J. 501
 Nkrumah, Francis K. 211, 574, 559, 890
 Nkrumah, Louis J. 327, 647
 Nkwengulila, Gamba 1054, 1055
 Nkya, Watoky M. M. M. 172
 Nobiya, Theresa 692
 Nobre, Mauricio L. 1061
 Nobrega, Priscilla F. 1061
 Noda, Jose A. 436, 438
 Nøddegaard, Louise 63
 Noedl, Harald 374, 87
 Noireau, François 738
 Noisakran, Sansanee 466
 Nolan, Thomas J. 1107
 Noronha, Antonio C. N.. F. 498
 Noronha, Elza 788
 Norris, Douglas E. 1060, 241, 591
 Nounougou, Diderot T. 181
 Nour, Bakri Y. M. 549
 Nourbakhsh, Shadi 71
 Novak, R.J. 599
 Novak, Robert J. 616
 Novoa, Jonathan 1107
 Nowosad, A. 382
 Nozaki, Tomoyoshi 384
 Nsobya, Sammuel L. -. 375
 Nuñez, Andrea 367
 Nuchprayoon, Surang 124, 820
 Nunes, Marcio 644
 Nunes, Márcio R. T. 1009, 962
 Nunez, Carmen 1104
 Nuñez, Luz 436, 438
 Nunomura, Wataru 854
 Nutman, Thomas B. 1019, 1020, 1021, 1022, 1023, 125, 351, 707, 709, 821, 435
 Nuwayri-Salti, Nuha 150
 Nwabueze, Nwabueze E. U. 430
 Nwachukwu, Chukwuemeka 67
 Nwakanma, Davis 231
 Nwaorgu, Onyekwere G. 723
 Nweke, Laz N. 78
 Nyirongo, Suzgo 308, 418
 Nzarubara, Bridget 343, 552, 717
 Nze Obiang, Pascal Christian 329
 Nzila, Alexis M. 193, 720
 Nzovu, Joseph G. 616
- O**
- O, Akanbi 561
 O' Brochta, David A. 695, 587, 910
 O'Connell, Amy E. 1108
 O'Connor, Linda-Lou 253
 O'Neal, Seth 1035
 O'Reilly, Terrence 670, 671, 676
 O'Rourke, Dorcas 964
 O'Rullian, Bill 701
 O., Olalubi A. 561
 Oakley, Miranda S. 974
 Obadofin, Michael 564
 Obaldia, Nicanor 1052, 335
 Obare, Peter 998
 Obi, Larry 266
 Obiesie, Nkolika 278
 Obiora, Ogechukwu E. 84
 Obrist, Brigit 29
 Occhialini, Lea T. 483
 Ochiai, R. L. 443
 Ochoa, Johanna 202
 Ockenhouse, Christian 215
 Odaibo, Alex B. 152
 Odera, James Sande 998
 Oduol, Frederick 21
 Oduola, Ayoade M. J. 187
 Oduro, Abraham 559, 890, 890
 Oduro, Rexford A. 211
 Offianan, Toure A. 194
 Ofula, Victor O. 285
 Ogata, Steven A. 983, 980, 1045
 Ogobara K., Doumbo 206
 Ogunbayo, Abayomi O. 161
 Ogundele, Sunday O. 722, 723
 Ogunkunle, Oluwatoyin O. 173
 Oguonu, Tagbo A. 85, 86, 423
 Oguonu, Tagbo M. 31
 Ogutu, Benhards 215
 Ogutu, Bernhards 770, 773, 775, 998
 Oh, Chang Mi 258
 Ohrt, Colin 770, 998
 Oishi, Kazunori 495
 Okada, Mami 384
 Okafor, Henrietta U. 85, 86, 31, 423
 Okamoto, Masahiko 500
 Okebe, Joseph 67
 Okech, Bernard A. 15
 Okeyo-Owuor, Joash 609
 Okomo, uduak 67
 Okoye, Patricia N. 584
 Olaya, Beatriz 555
 Oldland, William K. 611
 Oleinikov, Andrew V. 356
 Olivares, Marcela 109
 Oliveira, Giane A. 1101
 Oliveira, Sabrina B. 582
 Oliveira, Tricia M. 498
 Oliveira-Ferreira, Joseli 198
 Olivo, Angélica 55
 Olley, Benjamin 429
 Olliaro, Piero L. 306, 307, 718
 Olorunsogo, Olufunso O. 161
 Olsen, Sonja J. 1039, 49
 Olsen-Rasmussen, Melissa 636
 Olson, James G. 767
 Olson, Jimmy K. 40
 Olson, Ken E. 249, 593, 597, 725, 729, 918, 806, 960, 965
 Olson, Victoria A. 680
 Oluleye, Ibukun O. 186
 Olveda, Remigio 22, 631
 Olveda, Remigio M. 1070, 23, 24, 417
 Omara, Mildred 325
 Omoding, Nicholas 26
 Ong'echa, John-Michael 344, 352, 560, 997, 513, 208, 207, 156, 158
 Onofrio, Robert 326
 Onwuchekwa, Uma U. 1084, 1085, 698, 757
 Onyango, Clayton 285
 Onyeka, Preet I. K. 430
 Opara, A 501
 Ophorst, Olga 1051
 Opika-Opoka, Robert 520
 Opoku, Ernest 309
 Opondo, Dorothy A. 352
 Orago, Alloys S. S. 208, 352
 Orchard, Paul J. 520
 Orcutt, Andrew C. 1098
 Ore, Carlos V. 545
 Orejuela, Leonora 785
 Orejuela Aristizabal, Leonora 454
 Orelus, Nicolas 693
 Oria, Reinaldo B. 399, 782, 944
 Orillo, Beverly 983
 Orlandi, Palmer A. 453
 Orogade, Adeola 31, 423
 Orogade, A A. 85
 Orogade, Adeola A. 86
 Orogwu, Steve 78
 Orsolini, Paolo 431
 Ortega, Migdalis 827
 Ortega, Ynes 381, 624, 945
 Ortega, Ynes R. 948
 Ortiz, Gloria M. 315
 Ortiz, Jaime 1082
 Ortiz, Justin R. 756
 Osae, Christian T. 407
 Osborn, Frances R. 885
 Osei-Kwasi, Mubarak 956
 Oshira, Sandy 401
 Osisanya, Wemimo 723
 Oskam, Linda 1063, 1064, 403, 703
 Osoga, Joseph 998
 Osorio, Jorge E. 680
 Osorio, Lyda 202, 555, 879
 Osorio, Yaneth 506, 650
 Osuna, Antonio 269
 Oswald, Cameron 95
 Othoro Watta, Caroline 1101
 Otido, Julius 422
 Otieno, Michael F. 208, 352
 Otieno, Richard O. 156, 207, 208, 352, 513, 560, 997
 Otieno, Walter 773, 775
 Ottone, Catherine 516
 Ouari, Ali 724
 Ouattara, Amed 200
 Ouedraogo, and Jean Bosco 373
 Ouffouet, Fulgence 194
 Ould Brahim, Hamoud 210
 Ouma, Collins 156, 158, 207, 208, 352, 513, 560, 997
 Ouma, John H. 987
 Ouma, Yamo E. 208, 513, 997, 207
 Ounah, David 344
 Overstreet, Michael 1053
 Oweka-Onyee, James 325
 Owen, Robert 964
 Owino, Simon 894
 Oyama, T 465
 Oyo-Ita, Angela 67
 Oyo-Ita, Esu 67
 Oyugi, Mary 770
- P**
- Pa'au, Molisamoa 345
 Pach, Al 42
 Pachas, Paul 791, 792, 929
 Pachas Trujillo, Zulma 746
 Pacheco, M. 62
 Pacheco, Víctor 778, 779, 776
 Pack, Robert 42
 Packham, Andrea E. 383
 Paddock, Christopher D. 1065
 Padilla, Angel M. 970
 Paessler, Slobodan 637, 638, 639, 640, 681
 Page, A. 443
 Pahan, David 1063
 Painter, Heather J. 859
 Palacios, Juan F. 825
 Palatnik de Sousa, Clarisa 507
 Paliwal, Jyoti 867
 Palmer, Dupeh 801
 Pam, Sunday 564
 Pan, Weiqing 1096, 1097
 Pan, Xuegong 1096, 217
 Pandey, Akhilesh 667
 Pandey, Krishna 447
 Pandey, Krishna P. 446, 494
 Panichakul, Tasanee 1102

- Panoskaltis-Mortari, Angela 520
 Pantones, Pam 278
 Pape, Jean W. 496
 Paragas, Jason 957, 961, 963
 Paraguai de Souza, Edilma 507
 Pardo, Jorge 804, 979
 Pardo, Karim 792
 Parham, Leda 473
 Parija, Subhash C. 262, 51
 Parish, Lindsay A. 843
 Park, Gregory S. 520
 Park, Jae-Won 153
 Park, Junguk 819
 Parker, Michael D. 682, 633
 Parker, Tina M. 60
 Parker, William 966
 Parmakelis, Aris 916
 Parra, Rolando 1031
 Parrado, Antonio R. 1009
 Parzy, Daniel 329
 Pascale, Juan M. 823, 827, 958
 Pasetti, Marcela F. 283
 Passos, Sara T. 451, 1035
 Patel, Jigar J. 657, 653
 Patel, Mili 10
 Patel, Samir N. 162, 852, 893, 975
 Patel, Sheral S. 993
 Patrican, Lisa A. 940
 Patabhi, Sowmya 413, 52
 Pattanapunyasat, Kovit 151
 Pattanawong, Urassaya 898
 Patterson, Noelle B. 1050, 577, 899
 Pau, Maria-Gracia 1051
 Paul, Jaishree 260
 Paul, Levine 606
 Paul, Richard 30
 Pavon, Alequis 469
 Payet, Vincent 397
 Paz, Carlos 502
 Pearson, Julie 381, 624
 Peckham, Edward 587, 695
 Pedersen, Bonnie R. 323
 Pedersen, Erling M. 483
 Penali, Louis 334, 542, 543
 Penali, L 855
 Penali, Louis K. 194
 Peñaranda, Rosaura 436, 438
 Peniche, Alvaro 54
 Peniche, Alex G. 506
 Peppercorn, Amanda 1067
 Perdomo, Deisy 499
 Perez, Ana Beatriz 103, 101, 107, 108
 Pérez, Gerardo 114
 Perez, Kialing 745
 Perez, Maria Angeles 111
 Perkins, Douglas J. 156, 158, 207, 208, 344, 352, 491, 513, 560, 997
 Pernalet, Martha 862
 Perng, Guey Chuen 1010, 466
 Persson, Kristina 357
 Persson, Kristina E. M. 986
 Peruski, Leonard F. 1039, 49, 699
 Pesko, Kendra 811
 Peters, Clarence J. 662
 Peters, Ryan J. 477
 Petersen, Nadine 664
 Peterson, David S. 354
 Peterson, Kristine 380
 Peterson, Nathan A. 732
 Petiard, Florence 458
 Petrarca, Vincenzo 231, 589, 607
 Petras, J. M. 76
 Petri, William A. 380, 384, 622, 941
 Petridis, Michael 1012, 580
 Petritus, Patricia M. 565
 Peyton, David H. 540, 539
 Pfeiffer, Martin 282, 44
 Phalkey, Revati 307, 307, 718
 Phasuk, R 286
 Phiri, Kamija 88
 Piccinali, Romina V. 738
 Pichyangkul, Sathit 218, 566
 Picot, Stephane 1003, 876
 Pieniazek, Norman J. 1037
 Pierce, Emily 503
 Pierre, Amenold 69
 Pierro, Dennis J. 249, 960, 965
 Pillai, Dylan R. 333, 999
 Pimentel, Guillermo 60
 Pimgate, Chusak 472
 Pina-Aguilar, Raul 508
 Pinedo, Viviana V. 914, 568
 Pinkerton, Relana C. 949, 399, 782
 Pinkus, Geraldine 994
 Pinoges, Loretxu 1033, 700
 Pinto, Joao 607
 Piola, Patrice 419, 700
 Platt, Kenneth B. 1041
 Pleydell, D 715
 Plichart, Catherine 485
 Ploegh, Hidde L. 563
 Plotinsky, Rachel N. 37
 Plowe, Christopher V. 1100, 200, 308, 884, 892
 Pocaterra, Leonor A. 436, 438
 Poe, Amanda 190
 Polhemus, Mark 773, 775
 Polley, Spencer D. 988
 Pombi, Marco 1014, 229, 589
 Pompliano, David L. 1004, 530
 Ponlawat, Alongkot 603
 Ponnusamy, Loganathan 917
 Pool, Robert 14
 Popper, Stephen 1008
 Porco, Travis 316
 Porksakorn, Chantima 820
 Porras, Beatriz 555
 Porras-Pedroza, Beatriz 879
 Porter, Kevin R. 439, 457, 467, 804, 979, 104
 Posner, Gary H. 526, 652
 Postigo, Jorge 488
 Poudel, Shreekanta 121
 Povoas, Marinete M. 238
 Powell, Eugene E. 602
 Powell, Jeffrey R. 916
 Powell, Rhonda R. 620
 Powers, Ann M. 638, 960, 965
 Prado, Irina 106
 Pramanik, Soroj 1105
 Prapasiri, Prabda 699
 Prather, Donald M. 300
 Preis, Jack 271
 Premji, Zulfiqarali 755
 Prescott, Joseph 679
 Preston, Benjamin M. J. 219
 Pretell, Javier 393, 412
 Price, RN 159, 339, 719, 995
 Prichard, Roger K. 481
 Prieur, Delphine 329
 Prigge, Sean T. 869, 873
 Priotto, Gerardo 1033
 Pritchard, David 1109
 Provenza, Giovanni 77, 784
 Prudhomme, Wendy 998
 Puccia, Vincent 744
 Puella, Jose MI 90
 Pulungsih, Sri P. 443
 Punjabi, Narain H. 443
 Pupilampu, Naiki 65, 91
 Pupo, Maritza 101
 Purcell, Lisa A. 212
 Purcell, Robert 80
 Purfield, Anne 537
 Purh, Nancy 312
 Purisaca, Enrique 116
 Putaporntip, Chaturong 863, 898
 Putnak, J. R. 980
 Putnak, Robert 797, 984
 Putnam, John L. 7
 Putnam, Shannon D. 1088, 289, 443
- Q**
- Qadri, Firdausi 290, 291
 Qiu, Dongchuan 1073
 Qiu, J 715
 Qiu, Sumei 1096
 Quasie, Olga 407
 Quay, Charles 396, 45
 Queiroz, Jose W. 1061
 Queiroz, Nina M. G.. P. 498
 Quelal, Claudia 879
 Quetel, Julia 267, 269
 Quinn, Conrad 1067
 Quinn, Matthew 1095
 Quinnell, Rupert 1109
 Quiñones, Luz 468
 Quiñónez, Luis 879
 Quintana, Fernando 740
 Quirt, Ian 160, 166
 Qureshi, Shahida 48
 Qvarnstrom, Yvonne L. 385, 453, 758
- R**
- Rabaa, Maia A. 470
 Raccurt, Christian P. 623
 Raczniak, Gregory A. 65, 91, 574
 Radhakrishna, Suman 1038
 Radošević, Katarina 1051
 Rahme, Elham 1104
 Rahnama, A 1032
 Raikhel, Alexander S. 907, 909
 Rajakaruna, Rupika S. 551
 Rajapakse, Jayanthe 132
 Ram, Pavani K. 1086, 42
 Ramachandran, Vandana 221
 Ramadorai, Vishant 457
 Ramakrishna, Balakrishnan S. 41
 Ramberg, Frank 20
 Ramey, Kiantra I. 149, 851
 Ramey, Wanichaya N. 1042
 Ramirez, Josefina 644
 Ramirez, Jhon E. 694
 Ramirez-Sierra, Maria Jesus 397
 Ramos, Mary M. 468
 Ramzy, Reda M. R. 480
 Randle, Nadine P. 696
 Rangasamy, Velusamy 25
 Rani, Rekha 260
 Ranson, Hilary 17, 696
 Rao, Ramakrishna U. 348, 486, 706, 128
 Raoul, F 715
 Rare, Lawrence 33
 Rasgon, Jason L. 1012, 19, 580
 Rastogi, Rakesh 1079
 Ratanachuen, Woraphol 656
 Rathod, Pradipsinh 163
 Rathore, Dharmendar 332
 Raviprakash, Kanakatte 104, 457, 518
 Raxcacoj, Gabriela 825
 Raychaudhuri, Syamal 500
 Rayner, Jonathan 104
 Rayner, Julian C. 841, 843, 900
 Razaghian, A 1032
 Rea, Ivan 1031
 Reagan, Sarah 50
 Recusani, Franco 760
 Reddy, M V. R. 1025
 Redmond, Seth N. 234, 931
 Reed, Calvin B. 804, 979
 Reed, Holly 317
 Reed, Steven G. 500
 Reeder, John C. 33, 993, 357
 Reese, Sara M. 645
 Reeves, Will 80

A-16

Important Note: The number(s) following author names refers to the abstract number.

- Regadera, Javier 1004
 Regis, David P. 1050
 Regnery, Russell L. 680, 636
 Reid, Michael 170
 Reid, Mark 171
 Reiling, Linda 986
 Reinhardt, Brian 146
 Reis, Eliana A. G. 1009
 Reis, Mitermayer G. 1009
 Reiskind, Michael H. 459
 Reitstetter, Raven E. 405
 Reller, Megan E. 48
 Relman, David A. 1008
 Remais, Justin 1073
 Remich, Shon A. 770, 773, 775, 338, 998
 Remo, Allison M. 513
 Ren, Xiaoxia 1012, 580
 Renault, Cybele A. 493
 Rendi-Wagner, Pamela 985
 Rendón- Ramírez, Ruth 113
 Renuka, K. 288
 Reporter, Roshan 47, 701
 Reske, Sven Norbert 751
 Ressner, Roseanne A. 405
 Reuter, Stefan 713, 751
 Revathi, G 625
 Reyes-del Valle, Jorge 1090
 Reynolds, Kevin A. 869
 Reynolds, Mary 278
 Reza, Avid 676
 Rian, Sigrid K. 594, 930
 Ribas, Maria A. 442, 444, 74
 Ribeiro, Jose 1017, 1078
 Richards, Allen L. 361, 365
 Richards, Frank O. 1069, 347, 78
 Richards, Jane 347
 Richards, Stephanie L. 811, 921
 Richardson, Jason H. 7
 Richardus, Jan Hendrik 1063
 Richie, Nancy 204, 903
 Richie, Thomas L. 1050, 518, 577, 899, 1048, 204, 574, 903
 Richter, Daniel J. 326
 Rico-Hesse, Rebeca 727
 Riddle, Mark S. 289
 Riehle, Michael 20, 582
 Riehle, Michelle M. 1018
 Rientong, Somsak 49
 Riera, Celia 90
 Rifakis, Pedro M. 77, 784
 Rijnbrand, Cornelius 639
 Rim, Han-Jong 790
 Rimando, Agnes M. 865
 Rimoin, Anne W. 659
 Ringwald, Pascal 550, 876
 Ríos, Adan 823
 Ripoll, Carlos 644
 Riscoe, Michael 336
 Riscoe, Mike 337, 870, 872
 Rivard, Robert G. 405
 Rivera, Blanca E. 825
 Rivero, José 862
 Rizzo, Nidia 347
 Robert, Kizindo 553
 Roberts, Diane M. 1044
 Roberts, Jacquelin M. 145
 Robertson, Adam 948
 Robertson, Brett 170
 Robertson, Ginger A. 710, 818
 Robich, Rebecca M. 928
 Robinson, Alan S. 222
 Robinson, Belinda 783
 Robinson, Douglas A. 940
 Robinson, Jaimie 804
 Robinson, Sally 576
 Robles Flores, Martha 259
 Rocha, Crisanta 1094, 111, 367
 Rocha, Claudio 767
 Rocheleau, Thomas A. 120, 228
 Rockett, Kirk A. 211
 Rodkwamtook, Wutikon 445
 Rodpradit, P 370
 Rodpradit, Prinyada 371
 Rodrigo, W. W. Shanaka I. 105
 Rodrigues, Flávia G. 582
 Rodrigues, Laura 1103
 Rodrigues, Sueli G. 1009, 196
 Rodríguez, Beatriz 530
 Rodríguez, Ileana 77
 Rodríguez, Mary L. 389, 391, 712
 Rodríguez, Rosmari 106
 Rodríguez, Richard 1082
 Rodríguez, Silvia 390, 392, 393, 410, 411
 Rodríguez, Sueli 644
 Rodríguez, Silvia 712, 714
 Rodríguez-Morales, Alfonso J. 143, 442, 444, 62, 68, 74, 77, 784
 Rodríguez-Roche, Rosmari 100, 102, 369, 99
 Rodulfo, Hectorina E. 885
 Roehrig, John T. 475
 Roepe, Paul D. 185
 Roeper, Brooke A. 960, 965
 Rogers, Bill 890
 Rogers, William O. 211, 574
 Rogerson, Stephen J. 323, 357, 414
 Roghmann, Mary-Claire 1084
 Rohlinger, Eric M. 983
 Rojas, Edna 366
 Rojas, Elsy 436
 Rojas, Iliana 792
 Rojas, J 929
 Rojas, Yanina 791
 Rojas- Larios, Fabian 144
 Rojo Domínguez, Arturo 259
 Roldan, Luis 792
 Rollenhagen, Julianne E. 291
 Rollins, Sean M. 1067
 Romagosa, Cleofé 1001, 324, 674
 Romanos, Eduardo 530
 Romero, Gustavo 788
 Romero, Mirza 55
 Romero, Margarita 901
 Romero-Severson, Jeanne 612
 Romoser, William S. 257
 Roncal, Norma 335, 869, 873
 Roncal, Norma E. 871
 Roncalés, María 535, 169
 Roos, David S. 647
 Rosa A.P., Amelia Travassos d. 641
 Rosario, Delfina 106, 473
 Rosas, Lucia 687, 839
 Rose, Gregory W. 554
 Rose, Heather 176
 Rose, Robert C. 105, 1095
 Rosenberg, Charles S. 148
 Rosenblatt, John E. 176
 Rosenqvist, Einar 700
 Rosenthal, Andrew S. 526, 652
 Rosenthal, Philip J. 1004, 181, 305, 320, 341, 343, 375, 377, 552, 672, 673, 717, 896, 999, 373, 1000
 Ross, Linda 806
 Rossi, Cynthia A. 659
 Rossnagle, Eddie 356
 Rothman, A 370
 Rothman, Alan L. 1044, 800, 805
 Røttingen, John-Arne 700
 Rouamba, Noel 373
 Roux, Kenneth 564
 Rowe, Alexander K. 10, 1075
 Rowland-Jones, Sarah 1006
 Rowley, Wayne A. 1041
 Roy, Shantanu 622
 Roy Chowdhury, Rinku 615
 Rozmajzl, Patrick J. 361
 Ruangareerate, Toon 164, 861
 Rubiano, Luisa 555
 Rubio-Palis, Yasmin 795
 Ruiz, Didy 101
 Ruiz, Marilyn O. 938, 932
 Rulisa, Steven 874
 Rullas, Joaquín 1004
 Rumaseuw, R 719
 Ruprecht, Ruth M. 27, 954
 Rusev, Ivan 633
 Rush, Amy C. 816
 Russo, Susan 234, 931
 Ruxrungtham, Kiat 218
 Rwigyondo, Claude 875
 Rwakimari, John B. 305
 Rwegoshora, Theophil 483
 Ryan, Edward T. 1067, 290, 291
 Ryder, Kate L. 1070
 Ryder, Robert W. 32
 Ryu, Hye Sun 258
 Ryu, Seung-Ho 153

S

- Sa-ngasang, Areeerat 474
 Sabatelli, Lorenzo 1103
 Sabeti, Pardis C. 326
 Sacci, John B. 1048
 Sack, David A. 702
 Saeed, Osman K. Saeed. 549
 Saenz, Fabian E. 569
 Safeukui, Innocent 516
 Sagara, Issaka 1100, 856
 Sagay, Soloman 564
 Sagnon, N'Fale 229
 Sague, Miriam A. 956
 Sahebani, Nematallah -. -. 261
 Sahu, Priyadarshi S. 51
 Saint-Jean, Yvan 90
 Sakamoto, Hirokazu 1047
 Saksena, Rina 291
 Sakyi, Kodjo 398
 Salafsky, Bernard 25
 Salanti, Ali 551
 Salas, Alejandra 488
 Salas, Carola 545, 546
 Salas-Benito, Juan S. 1090
 Salasek, Michael 795
 Salazar, Belem 776, 778, 779
 Salazar, Jaime 791
 Salazar, Ximena 138
 Salazar-Bravo, Jorge 958
 Saldaña, Azael 823
 Saldarriaga, Omar A. 650
 Salika, Prasert 699
 Salimata, Konate 860
 Salmón-Mulanovich, Gabriela 275, 73, 92
 Sam-Yellowe, Tobili Y. 648
 Samake, Mariam 753
 Samake, Sibiri 502
 Samake, Sibiry 741
 Samyao, Blanca 825
 Samie, Amidou 266
 Sammons, Scott A. 279, 636
 Sampaio, Gabriel A. 1061
 Sampey, Darryl 277
 Samsi, Tatang K. 467
 Sanasuttipun, Wiwan 49
 Sanchez, Jose L. 133
 Sanchez, Jorge L. 133
 Sanchez, Ricardo 785
 Sanchez-Hidalgo, Lelia 389, 390, 391, 410
 Sánchez-Jiménez, Miriam M. 452
 Sanchez-Vargas, Irma J. 597, 725, 806
 Sandefur, Conner I. 376
 Sanders, Heather 806
 Sanders, Todd A. 1042
 Sanders-Lewis, Kolby 1037, 89
 Sandhu, Gurjinder S. 1083
 Sandoval, Clara 138
 Sandoval, M A. 1076

Important Note: The number(s) following author names refers to the abstract number.

A-17

- Sang, Dongpei 526
 Sang, Rosemary C. 924
 Sangare, Constance Souko 741
 Sangare, Djibril 1017
 Sangaré, L 866
 Sangsuk, Leelawadee 49
 Sangsuk, Leelaowadee 699
 Sanogo, Yibayiri O. 912
 Sanprasert, Vivornpun 820
 Santamaria, Cynthia 833
 Santamaria, Giovana 823
 Santana, Emidaly 106
 Santiago, Gilberto 468
 Santiago, Jose 292
 Santillan, Frida 1076
 Santivañez, Saul J. 712
 Santolalla, Meddly 546
 Santolamazza, Federica 607
 Santos, Carl P. 592
 Santos, Fatima 198
 Santos, Marlete Silva 788
 Santos, Terezinha J. 399
 Sanz, Sergi 1001, 324, 674
 Sarfo, Bismark Y. 851
 Sarpong-Nsiah, Margaret 1074
 Sarr, Demba 210
 Sarr, Moussa D. 30
 Sarr, Ousmane 326
 Sarracino, David 290
 Sarria, Magdeleine 792
 Sasi, Philip 720
 Sathe, Neeraj 982
 Satimai, Wichai 374
 Satoskar, Abhay 687, 839, 971
 Sattabongkot, Jetsumon 1047, 164, 532, 538, 861, 904
 Satti, Maria 769
 Saul, Allan J. 1100, 1098, 220, 578
 Saul, Lozano 606
 Saunders, Randy 1015
 Savage, Harry M. 250
 Sawanpanyalert, Pathom 474
 Sawasaki, Tatsuya 1047
 Sayang, Collins 718
 Scanfled, Dan 303
 Schaffner, Steve 326
 Schal, Coby 917
 Schallig, Henk D. 497
 Schallig, Henk D. F. H. 549
 Schellenberg, David 13, 14, 35, 764
 Schellenberg, Joanna 14, 35, 764
 Scherf, Artur 510
 Schlesinger, Jacob J. 105, 1095
 Schmid, Laura J. 821
 Schmidt, Kelsey M. 918
 Schmolke, Kathrin 108
 Schneider, Bradley S. 120
 Schneider, Dominique 488
 Schneider, David 596
 Schneider, Renate 69
 Schnorr, Daniel 69
 Schnupp, Carol P. 395
 Schoone, Gerard J. Schoone. 549
 Schotthoeffler, Anna 932
 Schousboe, Mette L. 551
 Schranz, Sabine 985
 Schuitema, Anja R. J. 403
 Schultsz, Constance 287
 Schulze, Alexander 29
 Schüpbach, Jörg 319
 Schwab, Anne E. 481
 Schwabe, Christopher 1081, 9
 Schwenkenbecher, Jan M. 130
 Scopel, Kézia K. 196
 Scott, Alan L. 677, 820
 Scott, James C. 316
 Scott, Thomas W. 372, 726, 245, 256, 370
 Sechan, Yves 485
 Seck, Yacine 977
 Seckova, Silvia 428
 Secor, W. Evan 969, 27, 947, 954
 Sedegah, Martha 518, 574, 577, 903
 Sedyaningsih, Endang 1088
 Seethamchai, Sunee 863
 Seguro, Antonio 81
 Sehdev, Paul 200
 Seidlein, L. v. 443
 Seino, Kathy K. 1046, 479, 815
 Sekou F, Traore 606
 Selanikio, Joel 692
 Sembiring, Masri 420
 Semenya, Amma A. 524, 840, 848
 Semnani, Roshanak T. 1021
 Sempertegui, Fernando 771
 Senda, James 1045, 983
 Sendagire, Hakim 654
 Sengul, Meryem S. 906
 Senn, Nicolas 58
 Senou, Martin Y. 194
 Seoh, Ju-Young 153
 Sepe, Daphne 252
 Serghides, Lena 975
 Serrano, Adelfa E. 880
 Setiabudi, Djatnika 467
 Seto, Edmund 1071
 Severson, David W. 1016, 246, 595, 612
 Sevilleja, Jesus Emmanuel A. D. 789, 949, 496
 Sewankambo, Moses 553
 Seydel, Karl 557
 Seydou, Doumbia 606
 Shah, Cyril 319
 Shah, Kaanan 930
 Shah, Naman K. 550
 Shahabuddin, Mohammed 1056, 1058, 601
 Shaheen, Hind I. 289
 Shahum, Andrea 428
 Shai-Kobiler, Ela 27, 954
 Shamovsky, Oleg 326
 Shamwol, Pierre 145
 Shanks, G. Dennis 195
 Shapiro, Theresa A. 526
 Sharafi, Roya 350
 Sharakhov, Igor 588
 Sharakhova, Maria 1017, 588
 Sharma, Y D. 377
 Sharp, Brian 1081, 8, 9
 Shaw, Roosevelt 1105
 Shearer, Todd 335
 Sherman, Jonathan M. 1082
 Shermukhamedova, Dilbar 961, 963
 Shi, Qifang 565, 901
 Shi, Ya Ping 300, 894
 Shiao, Shin-Hong 909
 Shililu, Josephat I. 235, 616
 Shimp, Richard L. 578
 Shin, Eun-Hee 1080
 Shin, Sang W. 907
 Shingatgeri, Vyas M. 541, 867
 Shirima, Kizito 13, 35, 764
 Shoemaker, Ritchie C. 82, 83
 Si, Yuanzheng 179, 61
 Siadati, Ahmad 71
 Siakwa, Mante 950
 Sibiri, Sissoko 860
 Sibley, Carol H. 193, 376, 544
 Sibomana, Isaie 398
 Sieba, Ibrahim 493
 Sieglaff, Douglas H. 909
 Sierra, Beatriz 103, 107, 108
 Sierra, Gloria M. 366
 Sigauque, Betuel 1001, 674
 Sigrid, Rian 606
 Sihuincha, Moisés G. 767, 608
 Siirin, M 1045
 Silva, Ana Maria 81
 Silva, Ana K. J. 1061
 Silva, Ana M. 796
 Silva, Javier 111
 Silva, Luciano K. 1009
 Silva, Maria 410
 Silva, Natal S. 196
 Silva, Sheyla 111
 Silva, Silvia 442, 444, 68, 74
 Silveira, Rita C. V. 498
 Silverman, David J. 1065
 Simanjuntak, Cyrus H. 443
 Simard, Frederic 1014, 229, 589, 913, 916
 Simmons, Cameron P. 1007, 1008, 1005, 1006
 Simmons, Kaneatra J. 686, 688, 330, 689
 Simmons, Monika 797
 Simon, François 663
 Simon, Jakob K. 283, 1084
 Simonsen, Paul E. 483
 Sims, Jennifer S. 214
 Sindermann, Herbert 1031
 Singer, Burton H. 11
 Singer, Darrell M. 133
 Singh, Balwan 1049, 198
 Singh, Kshipra 865
 Singh, Mrigendra P. 991
 Singh, Neeru 991
 Singh, Priti 204
 Singh, Prince Y. 265
 Singh, Sanjay 668, 846
 Singh, Shubhankar K. 415
 Singh, Upinder 384, 621
 Singla, L D. 265
 Sinha, Prabhat K. Sinha. 446, 494, 415, 447
 Sinishtaj, Sandra 526
 Sinnis, Photini 294
 Sipilanyambe, Naawa 525, 527, 675
 Sirichaisinthop, Jeeraphat 374, 532
 Sirinarm, Pokasem 49
 Sirisopana, Narongrid 445
 Sissako, A 866
 Sissoko, Ibrahim 741
 Sissoko, Mady 1100, 351, 856
 Sissoko, Mahamadou S. 1100
 Sitati, Elizabeth M. 118
 Sidthirasd, Anussorn 1039
 Sithisiprasasna, R 370
 Siydel, Karl 299
 Skarbinski, Jacek 10, 1075
 Skovmand, Ole 927
 Slemenda, Susan 1037
 Slike, Bonnie M. 1091
 Sloane, Lynne 176
 Slodkowitz-Kowalska, Anna 382
 Sloma, Cari R. 176
 Slotman, Michel A. 916
 Slutsker, Laurence 894
 Sly, P 159
 Smilkstein, Martin 336
 Smilkstein, Marty 337, 870, 872
 Smith, David C. 317
 Smith, David L. 200, 43
 Smith, Joe D. 296
 Smith, Joseph D. 327
 Smith, Kristin E. 223
 Smith, Kirsten S. 335
 Smith, Leia 296
 Smith, Philip L. 335
 Smith, R. 599
 Smith, Stephen C. 598, 692
 Smith, Thomas A. 887
 Smits, Henk 406
 Snider, Heidi 687
 Snowden, Karen F. 40
 Soares, Alberto M. 782
 Soares, Irene S. 196
 Sobral, Bruno 384
 Sobsey, Mark 318
 Sobsey, Mark D. 315
 Sochanta, Tho 908
 Sodahlon, Yao K. 482, 75
 Sogoba, Moussa 1100, 856
 Sogoba, Nafomon 1078

A-18

Important Note: The number(s) following author names refers to the abstract number.

- Solano, Sandra 55
 Solarczyk, P. 382
 Solberg, Victoria B. 395
 Soler, Maritza 366
 Solis, Angel T 90
 Solorzano, Nelson 1066, 791, 929
 Some, Coirentin Y. 229
 Somsri, Sangdoea 904
 Sonenshine, Daniel E. 3
 Sonetti, David 787
 Soni, Nishant 243
 Sorensen, William 489
 Sosa, Iris 468
 Soto, Giselle 1082
 Soto, Jaime 1031, 1031
 Soto, Katherine 886
 Soto, Paula 1031
 Souza, Estéfano A. 196
 Sow, Samba O. 1084, 1085, 698, 753, 757
 Sowunmi, Akintunde 187
 Sowunmi, Akin 858, 857
 Sparrowe, John 530
 Spear, Robert 1073
 Speare, Richard 743
 Speck, Roberto F. 319
 Spence, Jennifer S. 653
 Spencer, Lilian 499
 Sperança, Márcia A. 196
 Spichler, Anne 1087, 81
 Spielman, A. 599
 Spielman, Andrew 928
 Spillmann, Cynthia 5
 Spithill, Terry 832, 833
 Spithill, Terrance W. 212
 Springer, Amy 296
 Sreenivasan, Meera 380
 Sreeramouju, Pranavi 787
 Srikiatkhachorn, A 370
 Srikiatkhachorn, Anon 472
 Sriram, Rama 385
 Sriwichai, Sabaithip 374, 421
 Staalsoe, Trine 172, 889, 973
 Staedke, Sarah G. 672, 717, 1000, 341, 343, 552, 721
 Stancil, Jeffrey 694
 Stange-Thomann, Nicole 326
 Stanisic, Danielle 514
 Stansic, Danielle 155
 Staprans, Silvija 891
 Starnbach, Michael N. 563
 Stauber, Christine E. 315
 Steadke, Sarah G. 305
 Steel, Cathy 1023
 Steele, Lisa N. 27, 954
 Stefaniak, Maureen E. 899
 Stein, David 639
 Steinauer, Michelle L. 733
 Steinbach, Thomas 179
 Steisslinger, Vera 817
 Steketee, Richard 975
 Stephens, Peter W. 536
 Stern, Eric J. 50
 Steurer, Frank 1038
 Steurer, Francis 141
 Stevens, Warren 434
 Stevens, Yvonne Y. 500
 Stewart, Juarine 1105
 Stewart, V. Ann 576
 Stiles, Jonathan K. 149, 851, 991
 Stins, Monique F. 523
 Stinson, Eric O. 234, 931
 Stinson, Seth O. 931
 Stockelman, Michael G. 1052
 Stoeckle, Marcel P. 319
 Stolk, Wilma A. 350
 Storeygard, Adam 955
 Storlie, Patricia A. 838
 Straccini, Christine 832
 Streit, Thomas 349
 Streit, Tom 693
 Strobert, Elizabeth 847, 972
 Strobe, Clare 17
 Ströher, Ute 660
 Stromdahl, Ellen Y. 361, 97
 Strong, Jim 660
 Stroup, Suzanne 384
 Stuart, Michael D. 602
 Stubberfield, Lisa 854
 Stubbs, Janine 986
 Sturrock, Robert F. 627, 736
 Styer, Linda M. 730
 Su, Kua E. 129
 Su, Li 23, 24
 Su, Qin 821
 Su, Xin-zhuan 1076, 299, 571
 Su, Xinzhuan 548
 Suarez, Lilliana 536
 Suárez, Luis 767
 Suarez-Ognio, Luis 424, 568, 642, 791, 792, 93
 Suazo, Harold 368
 Subbanagounder, Ganesamoorthy 864
 Subekti, Decy 443
 Subramanian, Hemavathy 104, 804, 979
 Subramanian, Ramanand A. 587, 910
 Suh, Kathryn N. 554
 Sukprasert, Walailuk 474
 Sulaiman, Irshad M. 279
 Sulieman, Dhafir D. 750
 Sullivan, David 523
 Sultana, Shazia 48
 Sumba, Peter O. 895
 Summer, Andrea P. 95
 Summons, Jorge 823
 Sun, Jianxin 394
 Sun, Peter 157
 Sun, Tao 711
 Sun, Wellington 797, 801, 984
 Sun, Yanjie 966
 Sungpradit, Sivapong 124
 Supali, Taniawati 128
 Susanti, Ika 191
 Susapu, Melinda 1034, 252
 Sutherland, Colin J. 219
 Sutthiratanana, Saithip 1039
 Sutton, Patrick L. 897
 Suwandono, Agus 420
 Suwannachote, Nantawan 247
 Suwonkerd, Wannapa 247
 Suzan, Gerardo 958
 Suzanne, M 866
 Suzuki, Stephanie 544
 Suzuki, Tomohiko 132
 Swaminathan, Srirama V. 992
 Swan, Ken F. 476
 Swedberg, Göte 656, 654
 Swerdlow, David L. 402
 Sylla, Mariam 1084, 1085, 698, 757
 Sylla, Massamba 939
 Szatanek, Tomasz 601
 Sztein, Marcelo B. 892
-
- T**
- Takala, Shannon L. 200
 Takeo, Satoru 1047, 1048, 532, 903
 Talaat, Kawsar R. 435
 Talavante, M^a Angeles 528
 Talbot, Elizabeth A. 37
 Talbot, Jeffrey 349
 Taleo, George 191
 Talisuna, Ambrose O. 305, 721
 Tall, Adama 30, 977
 Tall, Koureichi 502
 Talla, Idrissa 30
 Tallima, Hatem A. M. 66, 731
 Tallo, Veronica 631
 Tally, John 768
 Tamang, Leena 382
 Tamayo, Pablo 303
 Tambini, Moises 752
 Tambo, E 858
 Tamboura, Boubou 753
 Tammaing, Cindy 878
 Tamoufe, Ubald 659
 Tamrat, Abiy 425
 Tan, John C. 657
 Tan, Ratna I. 439
 Tang, De-chu 1052
 Tangpukdee, Noppadon 992
 Tanner, Marcel 13, 14, 319, 35, 764, 887
 Tannich, Egbert 622
 Tanser, Frank 8
 Tanyuksel, Mehmet 941
 Tao, Jing 648
 Tapia, Milagritos D. 1084, 1085, 698, 753, 757
 Tapia, Rafael 116
 Taquiri, Carmen 948
 Tarafder, Mushfiqur R. 631
 Tarazona, Augusto 791
 Tarleton, Jessica L. 148, 970
 Tarleton, Rick L. 147, 148, 505, 684, 970
 Tarongka, Nandao 1034
 Tauber, Erich 984, 985
 Taylor, Charles E. 1059, 594, 930
 Taylor, Terrie E. 340, 884, 557, 994
 Taylor, Walter (Bob) R. J. 306, 307
 Teates, Kathryn S. 756
 Teclaw, Robert 278
 Tediosi, Fabrizio 434
 Teixeira, Maria G. 1009
 Teixeira, Rosangela 735
 Tekwani, Babu L. 865
 Telang, Aparna 922
 Telford, Sam R. 360, 455
 Tembe, Elisa 1001
 Temesvari, Lesly A. 620, 942
 Teopipithaporn, Suriya 87
 ter Kuile, Feiko O. 88, 300
 Terajima, Masanori 1044
 Terashima, Angelica 735
 Tesh, Robert B. 641, 1045, 638
 Tetteh, Kevin K. A. 988
 Tettey, Yao 851
 Thailayil, Janis 222
 Thaisomboonsuk, Butsaya 472, 803, 370
 Thakur, Chandeshwar P. 626
 Thammapalo, S 370
 Thao, Le Thi Thu 1008
 The Tai, Diep 1086
 Thea, Donald M. 432
 Theander, Thor G. 172
 Theisen, Michael 172
 Thera, Mahamadou A. 1100, 200
 Thera, Mohamadu A. 892
 Thermoziar, Kerline 134
 Thi Phi La, Tran 1086
 Thi Phong Lan, Nguyen 1086
 Thibodeaux, Brett A. 475
 Thiem, Vu Dinh 43
 Thillier, Laurence 718
 Thimasarn, Krongthong 1079
 Thomas, Alan W. 219
 Thomas, Stephen J. 371, 797
 Thompson, Benedicta 309
 Thompson, Meghan R. 399
 Thompson, Peter A. 309
 Thorat, Swati 519
 Thorne, Michael 980
 Thorne, Mike 983
 Thornton, George B. 1101
 Thuma, Philip 241, 310
 Thuma, Phil 755
 Thuma, Philip E. 853
 Thumar, Bhavin 1094, 981
 Tia, Taweesak 898
 Ticona, Eduardo 1082
 Tidwell, Richard R. 537

- Tierney, Eveline 1096, 1097
 Timiryasova, Tatyana 807
 Ting, Edmund 797
 Ting, Li-Min 294
 Tinto, Halidou 373, 875
 Tisch, Daniel J. 346
 Tjaden, Jeffrey A. 804, 979
 Tjitra, E 159, 339, 719, 995
 Toledo, Julia 1031
 Tolouei Semnani, Roshanak 1019
 Tomas, Patricio 832
 Tomasik, Zuzana 319
 Tongkong, Dokruk 374
 Tongren, Jon Eric 203, 991
 Tonnetti, Laura 264
 Topalis, Pantelis 234, 931
 Torgerson, Paul 716, 715
 Torii, Motomi 1047
 Toro, Jesús 776, 778, 779
 Torres, Daricel 267, 268, 269
 Torres, Jose 53
 Torres, Miguel 1081
 Torres, Pedro 169, 535
 Torrez, Miguel 9
 Tosti, Christina L. 992
 Touré, A 331
 Touré, Mahamoudou B. 594, 606
 Toure, Ousmane 379
 Toure, Walentchin 194
 Toure-Ndouo, Fousseyni S. 154, 976
 Tovar, Marco 1082
 Tovar, Manuel 366
 Tran, Chau N. 1005
 Tran, Dat V. 465
 Tran, Tuan M. 198
 Traore, Arouna 493
 Traore, Abdel K. 125, 351, 484
 Traore, Abdoulaye M. 1100
 Traore, Oumar B. 379
 Traore, Pierre 502
 Traore, Sekou F. 741, 1078, 923, 926, 1017, 125, 351, 484, 594
 Trapaidze, Nino 384
 Travassos da Rosa, Amelia P. A. 638, 1045
 Travi, Bruno L. 506, 650
 Trijani, 443
 Trindade, Giliane 636
 Tripathi, Abhai 523
 Tripet, Frederic 397
 Troia, Giuliana 1036, 431
 Trongnipatt, Namtip 164, 861
 Trono, Karina 1043
 Troughton, Danielle R. 362
 Troyo, Adriana 613
 Trung, Ho Dinh 908
 Tsamo, Etienne 181
 Tsang, Victor 410, 413
 Tsang, Victor C. W. 392, 393, 409, 52
 Tsetsarkin, Konstantin A. 809
 Tshetu, Antoinette 32
 Tsoka, Joyce M. 558
 Tsuboi, Takafumi 1047, 1048, 532, 903
 Tsuji, Moriya 1051
 Tsukayama, Pablo 142
 Tu, Zhijian J. 1015, 583, 906
 Tucker, Brad J. 1041
 Tulyanon, Somchit 421
 Tumwesigye, Nathan 321
 Tungtaeng, Anchalee 335, 538
 Turell, Michael J. 682
 Turrentine, Jake E. 147
 Tuyen, Luc Nguyen 1109
 Twesigye, Rogers 419, 700
 Tweyongyere, Robert 26
 Tzipori, Saul 266
- U**
- Ubalee, Ratawan 164, 861
 Ubeid, Cristina 644
 Uda, Kouji 132
 Udhayakumar, Venkatachalam 188, 190, 203, 300, 894, 975, 991
 Udoji, Georgina 78
 Udomsangpetch, Rachanee 1047, 1102, 151, 898, 904
 Udujih, Udujih o. S. 430
 Udupa, Venkatesha 541, 867
 Ueberheide, Beatrix 21
 Ullmann, Amy 2
 Ulukanligil, Mustafa 941
 Umaru, John 1069
 Umeh, Sarah I. 581
 Umurzakov, Shavkat 961, 963
 Unger, Alon 1035, 57, 788
 Unger, Maria 232
 Unnasch, Emily A. 605
 Unnasch, Thomas R. 253, 293, 605, 611
 Ur-Kowitchai, Charn 87
 Urbina, MaryPaz 22
 Urdaneta, Ludmel 795, 862
 Usuga-Silva, Luz Y. 452
 Uthaimongkol, Nichapat 421
 Utz, Gregory C. 275
 Uyeki, Timothy M. 1088, 756
- V**
- Vaidya, Akhil 859
 Vaillant, Michel 306, 307
 Valadkhani, Zarintaj 837
 Valbuena, Gustavo 785
 Valda, Luis 1031
 Valencia, Anais 204
 Valencia, Teresa 1083
 Valenzuela, Jesus G 741, 3
 Valera, Manuel 139
 Valerio, Laura 256
 Valiyaveettil, Manojkumar 511
 Vall, Idoumou O. M. 663
 Van Boxlaer, J 542
 Van Cauteren, Marc 992
 van Cleeff, Maarten R. A. 703
 Van Dam, Govert 734
 van der Heyde, Henri 209
 van der Meide, Wendy F. 497
 van der Sluis, Allard 497
 van Dijk, Janneke 310, 853
 Van Dyke, Melissa K. 1072, 951
 Van Dyken, Meg 925
 van Eijk, Annemieke 975
 Van geertruyden, Jean-Pierre 322
 Van Gessel, Yvonne 984
 van Gool, Tom 497
 van Herp, Michel 425
 Van Miert, Sabine 531
 Van Sach, Nguyen 1086
 van Thiel, Pieter P. A. M. 497
 van Vugt, Michele 497
 Vanasco, Norma 80
 VandeBerg, John L. 834, 1027
 Vanden Eng, Jodi 300
 Vanderberg, Jerome 579
 vanEkeris, Leslie 16
 VanGordon, Gail 47, 701
 VanHook, Robert 435
 Vaniscotte, A 715
 VanKirk, N. 292
 Vanlandingham, Dana L. 809
 Varatharajalu, Ravi 824
 Varela, Marie Louise 655
 Vargas, Maria Jose 1094
 Vargas, Miguel 259
 Vasconcelos, Pedro F. C. 1009, 196, 962, 644
 Vasquez Camargo, Fabio 824
 Vatansever, Zati 957
 Vatsan, Ramjay 204
 Vaughan, Jefferson A. 121
 Vaughn, David W. 274
 Vazquez, Susana 100, 101, 469
 Vazquez-Prokopec, Gonzalo M. 5, 739
 Vega, Joel 880
 Velasco, Sofia 55
 Velasquez, Janette 388
 Véliz, Frank 778
 Venkatesan, Meera 19
 Vennervald, Birgitte J. 26
 Ventosilla, Palmira 1066
 Verastegui, Manuela R. 388, 714
 Vergara, Katherine C. 1083
 Vergne, Edgardo 802
 Verhaagh, Sandra 1051
 Verhave, Jan 793
 Verma, Neena 447
 Vernazza-Licht, Nicole 718
 Vernick, Kenneth D. 1018, 21
 Veshiyi, Judith M. V. 175
 Viall, Abigail 349
 Viallon, Jérôme 485
 Viboud, Cecile 313
 Vidal, Carlos E. 886, 897, 1083, 694, 767
 Vidal, Jaume 169, 535
 Viebig, Nicola K. 510
 Vieira, Carlos M. G. 399, 949
 Viera, Sara 528, 529, 530
 Viguilla, Vic 756
 Villalobos, Iris 366
 Villalta, Fernando 330, 686, 688, 689
 Villegas, Elci 614
 Villegas, Leopoldo 188, 776, 777, 778, 779, 862
 Villinski, Jeffrey T. 91
 Vince, Mary A. 97
- W**
- Wa-Shija, Robert 14
 Wabwire-Mangen, Fred 305
 Wade, William 291
 Waggoner, Skye 326
 Wahl-Jensen, Victoria 284
 Waindi, Eliud N. 156
 Waitayakul, Amornrat 904
 Waitumbi, John 215
 Walker, David 785
 Walker, Edward 604, 691, 927, 600, 932, 938
 Waller, Karena 854
 Waller, Lance A. 1026
 Wallis, Teresa R. 756
 Walzl, Gerhard 1106
 Wamae, N 625
 Wang, Gordon 1045
 Wang, Huey-Ching H. 263
 Wang, Qiang 1097
 Wang, QiZhi 632
 Wang, Q 715
 Wang, Shrijie 887
 Wang, Tongmin 648
 Wang, Wendy 296
 Wangsasaputra, Ferry 443
 Wanionek, Kimberli A. 981
 Wanke, Christine 771
 Wannemuehler, Kathleen 50
 Waramori, G 159, 995
 Warashina, Wes 401
 Ward, Brian 499, 832, 833
 Ward, Leigh 783
 Ward, Steve 180
 Ward, Steve A. 308, 418
 Ware, Lisa 576
 Warhurst, David C. 189
 Warke, Rajas 798, 799, 800
 Wasfy, Momtaz O. 60
 Wasieloski, Leonard P. 957
 Watanabe, Risa 532

A-20

Important Note: The number(s) following author names refers to the abstract number.

- Watanabe, Yoshiya 132
 Waters, Norman C. 869, 871, 873
 Watson, Julie 80
 Watson, Wes 364
 Watts, Douglas 1045
 Weaver, Scott C. 637, 638, 681
 Webster, Joanne 1068
 Weedall, Gareth D. 219
 Weeks-Levy, Carolyn 980, 1045, 983
 Wegner, Mark 278
 Weidanz, William 209
 Weil, Gary 128, 1034, 348, 480, 486, 704, 706, 816
 Weina, Peter 167, 178, 766, 775, 338, 61, 763, 765, 768, 830, 831
 Weiss, Günter 853
 Weiss, Mitchell G. 199
 Weiss, Walter 204, 903, 577
 Wellem, Thomas E. 295, 379, 996
 Wellikoff, Adam 744
 Welter*, Brenda H. 620
 Wenink, Emily C. 658
 Were, Tom 156, 207, 208, 344, 352, 513, 560
 Wesson, Dawn 917, 476, 602, 924, 933
 Westbrook, Catherine J. 19
 Wetzler, Lee 969
 Wheeler, Emily 932
 White, A. C. 1107
 White, Bradley J. 1014, 18
 White, Michael J. 317
 White, Nicholas J. 151
 White, Tacey E. 182
 White Jr, A Clinton 263
 Whitehead, Stephen 982, 981
 Whitehouse, Chris A. 957
 Whitlock, Darcy 789
 Whitt, Parker B. 602
 Whitty, Christopher 1000
 Wickremasinghe, Susiji 132
 Widjaja, Susana 467
 Widner, Stephanie 983
 Wiegand, Roger 326
 Wig, Naveet 288
 Wikel, Stephen K. 1
 Wilairat, Prapon 183
 Wilairatana, Polrat 992
 Wilcox, Bruce A. 401
 Wilkerson, Richard C. 238
 Wilkins, Elizabeth 586, 598
 Wilkins, Patricia 50
 Williams, Arthur 1105
 Williams, Francis T. 1050
 Williams, Gail M. 711
 Williams, Holly A. 670, 671, 676
 Williams, Hywel C. 1109
 Williams, Jeffrey F. 292
 Williams, Jeff T. 834
 Williams, Lucia 225
 Williams, Steven A. 483, 705
 Williams, Tom 517
 Williams, Thomas 986
 Williams-Blangero, Sarah 1027
 Williamson, Phillip C. 97
 Willis, Derek 11
 Wills, Bridget 1006
 Wilmot, Abba 398
 Wilson, Alan 734
 Wilson, Marianna 1037, 89
 Wilson, Mary 990
 Wilson, Michael D. 1074, 211, 396, 398, 45, 65
 Wilson, Mary E. 649, 685, 79, 838
 Wilson, Mark L. 1072, 34, 883, 951
 Wilson, Nana 851
 Wilson, Russell 277
 Wilson, Ron 936
 Wimonwattrawatee, Theera 87
 Wimsatt, Ashley 503, 835
 Winstanley, Peter 180
 Winstanley, Peter A. 308, 418
 Winston, Carla A. 10
 Winter, Rolf 336, 337, 870, 872
 Winzeler, Elizabeth A. 221, 303
 Wiredu, Edwin K. 851
 Wirth, Dyann F. 187, 214, 326, 328, 563
 Wirtz, Robert 676, 934
 Withers, Mark 215
 Withum, David 468
 Woda, Marcia L. 798
 Wofford, Taylor 50
 Wohlhueter, Robert M. 279
 Wolf, Christian 185
 Wolfe, Nathan D. 659
 Wölfel, Roman 44, 664
 Won, Kimberly 1037
 Wong, Mayee 401
 Wong, Teri 1045, 980, 983
 Wongjindanon, Wanna 49
 Wonjindanon, Wanna 1039
 Woo, Jonathan M. 673
 Wood, Chloe L. 576
 Wooden, Jason M. 376
 Wootton, Dan 180
 Worley, David 292
 Wortmann, Glenn 146, 416
 Wu, HaiWei 632
 Wu, Shuenn-Jue L. 457
 Wu, Yimin 578
 Wuryadi, Suharyono 420, 439
- X**
- Xacur-Garcia, Fiona 508
 Xhaja, Kris 798
 Xi, Zhiyong 907
 Xia, Ai 588
 Xiang, Hong 530, 864
 Xianli, Jia 1102
 Xiao, Lihua 381, 624, 625, 945, 948
 Xiao, Shu-Yuan 641
 Xie, Lisa 178
 Xie, Lisa H. 174
 Xu, Fangling 641
 Xu, Jiannong 1018, 21, 226
 Xu, JinMei 632
 Xu, Lun 217
 Xu, Lili 522
 Xu, Longqi 790
 Xu, MingXing 632
 Xu, Ning 917
 Xuan Bang, Bui 1086
 Xue, Yongkang 594, 930
- Y**
- Yabar, Carlos 116
 Yabsley, Michael 364
 Yalcoue, Daniel 351
 Yalcouye, Daniel 1100
 Yan, Guiyun 1057, 609
 Yancey, Linda S. 263
 Yang, Changhong 1073
 Yang, Liyan 1044
 Yang, Wenli 945
 Yang, Yu R. 711
 Yankson, Kwabena 950
 Yano, Hiroko 132
 Yanoviak, Stephen 694
 Yanow, Stephanie K. 212
 Yao, Chaoqun 649, 838
 Yapi, Jean D. 194
 Yaro, Alpha 923, 926, 240
 Yasmin, Tabassum 131, 742
 Yasunami, M 465
 Yatawara, Lalani 132
 Yates, Terry 958
 Ye, Chunyan 679
 Yearick, Kimberly S. 185
 Yeboah, Kwame 749
 Yeka, Adoke 721
 Yeo, TW 159, 339, 995
 Yeom, Joon-Sup 153
 Yi, Poravuth 550
 Yi, Pedro 735
 Yi-Xun, He 25
 Yilmaz, Hasan 941
 Yimamnuaychoke, Nongnuch 164
 Yingst, Samuel 633
 Ylostälo, Joni J. 304
 Yocupicio-Monroy, Martha E. 794
 Yohn, Christopher 562
 Yohn, Christopher T. 882
 Yohou, Kevin 194
 Yongkang, Xue 606
 Yongvanitchit, Kosol 218, 566
 Yoon, In-Kyu 146
 Yori, Pablo 1083
 Yoshino, Timothy P. 732
 Young, Carolyn 1037
 Young, John 1067
 Yourick, Debra L. 335
 Ypil-Butac, Charity Ann 803
 yu, guowei 201
 Yu, Jemaima 22
 Yuan, Jingyu 217
 Yue, Xin 611
 Yukich, Joshua 8, 434
 Yun, Nadezhda 637
 Yun, Nadezhda E. 639, 640, 681
 Yun, Yun E. 638
 Yuna, Hamissi 13
 Yusianto, Andi 420
 Yusuf, Bidemi 429, 722
 Yuwono, Djoko 467
- Z**
- Zacks, Michele A. 637, 638, 681
 Zaidenberg, Mario 5
 Zaidi, Anita K. M.. 48
 Zainoun, Joanne 326
 Zaks, Laurel 312, 437
 Zaldivar, Yamitzel 823
 Zamalloa, Hernan 461
 Zamora, Elvira 694
 Zarei, Z 1032
 Zavala, Fidel 1053, 353
 Zavala, Josue 740
 Zduniak, P 382
 Zea-Flores, Guillermo 347
 Zeidner, Nordin 2
 Zellweger, Michael J. 1029
 Zeng, Yong 628
 Zepeda, Orlando 846
 Zerom, Mehari 434
 Zevallos, Karine 1083
 Zhang, Chunlin 371
 Zhang, Jing 174, 178, 179
 Zhang, Jun 708
 Zhang, Lyna 300
 Zhang, Qiong 786
 Zhang, Si-Ming 628
 Zhang, Wen 1096
 Zhang, Yanling 578
 Zhang, Yanping 592
 Zhang, YuanYuan 632
 Zhao, Bangti 801
 Zhao, Wan-Qian 492
 Zhao, Weiguo 650
 Zhioua, Elyes 6
 Zhong, Bo 1073
 Zhou, Goufa 609
 Zhou, Hong 1098
 Zhou, Lei 592
 Zhou, MeiHong 632
 Zhou, Yaling 146
 Zhou, Yingyao 303
 Zhou, Zhiyong 190

Important Note: The number(s) following author names refers to the abstract number.

A-21

Zhu, Daming 220, 578
Zhu, Jianzhong 512
Zhu, Jack 821
Zhu, Shenjun 492
Zhuo, Tianle 850
Zijlstra, Edward 308
Zimic, Mirko 1082
Zimmerman, Michael 284
Zimmerman, Peter 323, 562,
1102, 155, 252, 33, 377, 378,
514, 881, 882
Zimmerman, Robert H. 934
Zoghbi, Normig 862
Zollner, Gabriela E. 7
Zongo, Issaka 373
Zorko, Nicholas 971
Zou, Xiaoyan 221
Zwetyenga, Joanna 718

